

University of Massachusetts Medical School

eScholarship@UMMS

GSBS Dissertations and Theses

Graduate School of Biomedical Sciences

1996-05-16

Neuroprotection and Neurotransplantation Strategies in Models of Parkinson's Disease

Wendy R. Galpern

University of Massachusetts Medical School

Let us know how access to this document benefits you.

Follow this and additional works at: https://escholarship.umassmed.edu/gsbs_diss



Part of the [Nervous System Commons](#), [Nervous System Diseases Commons](#), [Organic Chemicals Commons](#), [Pharmaceutical Preparations Commons](#), and the [Therapeutics Commons](#)

Repository Citation

Galpern WR. (1996). Neuroprotection and Neurotransplantation Strategies in Models of Parkinson's Disease. GSBS Dissertations and Theses. <https://doi.org/10.13028/vc6v-8y83>. Retrieved from https://escholarship.umassmed.edu/gsbs_diss/143

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in GSBS Dissertations and Theses by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.

**NEUROPROTECTION AND NEUROTRANSPLANTATION
STRATEGIES IN MODELS OF PARKINSON'S DISEASE**

A dissertation presented

by

WENDY R. GALPERN

Submitted to the Faculty of the University of Massachusetts Graduate School of
Biomedical Sciences, Worcester
in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

May 16, 1996

**NEUROPROTECTION AND NEUROTRANSPLANTATION
STRATEGIES IN MODELS OF PARKINSON'S DISEASE**

A dissertation presented

by

WENDY R. GALPERN

Approved as to style and content by:

Craig F. Ferris, Chair of Committee

Susan Gagliardi, Member of Committee

Daniel A. Pollen, Member of Committee

Suzanne Roffler-Tarlov, Member of Committee

Ole Isacson, Dissertation Mentor

Thomas B. Miller, Jr., Dean of the
Graduate School of Biomedical Sciences

Program in Biomedical Sciences

May 16, 1996

ACKNOWLEDGEMENT

There are many individuals who have been instrumental in achieving the work presented in this thesis. In particular, I would like to thank my thesis advisor Dr. Ole Isacson for his insight and guidance. He has provided an outstanding learning environment and excellent training which has enabled me to pursue my scientific interests and goals. In addition, I would like to extend my sincere appreciation to Dr. Xandra Breakefield for kindly allowing me to work in her laboratory and to Dr. Flint Beal for a fruitful collaboration.

I would also like to acknowledge the past and present members of the Neuroregeneration Laboratory for their support and contributions. I am especially grateful for the opportunity to have worked with Lindsay Burns, Terry Deacon, David Frim, Philippe Hantraye, Peyman Pakzaban, Stephen Tatter, and Tara Uhler who all generously shared their time and knowledge. Many thanks to Celeste LeBlanc and Sandra Pohlman for their kind efforts and assistance.

I wish to thank Dr. David Standaert who has greatly enriched my training by sharing his clinical experience, and I want to express my gratitude to Anna-Liisa Brownell, Iris Chen, Bruce Jenkins, Miguel Sena-Esteves, and John Yu for their valuable contributions.

A special thank you to my family for their love and encouragement.

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic cell death in the substantia nigra pars compacta (SNc) and dopamine (DA) depletion in the striatum. Current pharmacological treatments are aimed at the replacement of striatal DA via the administration of levodopa. While this therapy is beneficial initially, long-term treatment is associated with significant side effects, and disease progression continues. The present experiments investigate neuroprotective and neurotransplantation strategies as alternatives to palliative pharmacologic treatments:

The optimal therapeutic approach to neurodegenerative diseases would be to protect against cell death and prevent disease progression. PD is well-suited for such neuroprotective strategies as primarily one cell population is affected in this disorder. Neurotrophic factors (NTFs) have been identified which support dopaminergic neuronal survival *in vitro*. In the present studies, the neuroprotective effects of the neurotrophin brain-derived neurotrophic factor (BDNF) have been evaluated in a 1-methyl-4-phenylpyridinium (MPP+) model of substantia nigra (SN) degeneration. BDNF-secreting fibroblasts were implanted dorsal to the SN prior to the infusion of the mitochondrial complex I inhibitor MPP+. Subsequent histological analysis demonstrated that BDNF is able to attenuate MPP+ induced dopaminergic cell loss in the SNc. Moreover, neurochemical evaluation demonstrated that BDNF is able to enhance DA levels in the remaining SN neurons in this same paradigm.

The cause of cell death in neurodegenerative diseases likely involves the interaction of mitochondrial impairment, excitotoxicity, and oxidative stress. In

order to evaluate the mechanism of NTF-mediated protection, the ability of nerve growth factor (NGF) to attenuate the production of the oxidant peroxynitrite was evaluated in a model of mitochondrial impairment. NGF was found to decrease the production of 3-nitrotyrosine, the product of peroxynitrite mediated tyrosine nitration. Thus, NTF-mediated neuroprotection may act in part by decreasing reactive oxygen species and oxidative stress.

At present, neuroprotective therapies are not clinically available. An alternate therapeutic approach to PD is the replacement of striatal DA and reconstruction of synaptic circuitry via the intrastriatal transplantation of fetal dopaminergic neurons. Current transplantation protocols using human fetal tissue are constrained by limited tissue availability. In order to investigate an alternate cell source for the treatment of PD, fetal porcine dopaminergic neurons were implanted into the DA depleted striatum of 6-OHDA lesioned rats. Amphetamine-induced rotational recovery was monitored, and graft survival was evaluated 19 weeks after grafting. In immunosuppressed rats, porcine dopaminergic neurons were found to attenuate rotational deficits and extensively reinnervate the host striatum.

The neuroprotective effects of BDNF suggest that NTFs may be important mediators of dopaminergic neuronal survival and function in the adult brain. However, several conditions including appropriate dosage and delivery need to be determined before clinical applications may be achieved. As an alternative to neuroprotection, neurotransplantation not only restores striatal DA but also reconstructs the synaptic circuitry of the basal ganglia. The finding that porcine dopaminergic neurons survive with in adult host brain, reinnervate the DA

depleted striatum, and mediate functional recovery suggests that porcine DA neurons may serve as an alternate cell source for transplantation in PD.

TABLE OF CONTENTS

CHAPTER I: Introduction	1
Parkinson's disease	1
Clinicopathological features	1
Epidemiology	2
Etiology and pathogenesis	3
Functional organization of the basal ganglia and the role of dopamine	6
Pharmacological treatment strategies	9
Surgical treatment strategies	11
<i>Functional neurosurgery</i>	11
<i>Neurotransplantation</i>	12
Animal models of Parkinson's disease	15
6-Hydroxy-dopamine	15
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	17
Neurotrophic factors	19
Mechanisms of cellular injury and neurotrophic factor interventions	19
Role of neurotrophic factors	22
Classes of neurotrophic factors and regional expression	23
<i>Neurotrophins</i>	23
<i>Other neurotrophic factors</i>	23
Specificity and distribution of neurotrophic factor receptors in the adult brain	24
<i>Neurotrophins</i>	24
<i>Other neurotrophic factors</i>	27
Dopaminergic neurotrophic factors	28
Transplantation of fetal dopaminergic neurons	31
Initial transplantation experiments	32
Important variables in cell transplantation	32
<i>Donor age</i>	32
<i>Implantation site</i>	33
<i>Number of cells</i>	33
<i>Specificity of tissue</i>	34
Functional aspects of grafted dopaminergic neurons	34
<i>Behavioral studies</i>	34

<i>Neurochemical assessment of grafted neurons</i>	35
<i>Graft afferent and efferent connections</i>	37
<i>Restoration of lesion induced changes at the cellular level</i>	37
Xenotransplantation of fetal dopaminergic neurons	38
Immunological considerations for neurotransplantation	39
<i>Basic immunology</i>	40
<i>Immunologic considerations in CNS</i>	41
<i>Allografting</i>	42
<i>Xenotransplantation</i>	44
<i>Immunosuppressive agents</i>	45
 Aims of present studies	 47
 Chapter II: Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat	 48
Abstract	48
Introduction	49
Materials and methods	50
Genetic engineering and selection of a BDNF-secreting fibroblast cell-line	50
Co-culture of BDNF-secreting fibroblasts with fetal ventral mesencephalic neurons	53
Cell preparation and implantation in substantia nigra degeneration model	53
Morphometric procedures	57
Statistical analysis	57
Results	57
BDNF-secreting fibroblasts	57
Striatal infusion of MPP+ and SNc degeneration	58
Effects of BDNF-secreting grafts on SNc cell survival	61
Discussion	67

CHAPTER III: Cell-mediated delivery of brain-derived neurotrophic factor enhances dopamine levels in an MPP+ rat model of substantia nigra degeneration	71
Abstract	71
Introduction	72
Materials and methods	74
Construction of BDNF-secreting fibroblast cell line	74
Northern blot analysis	75
Enzyme linked immunoassay	75
Cell preparation, surgical implantation, and MPP+ lesion procedures	76
Neurochemical analysis and histology	79
Statistical analysis	79
Results	80
BDNF mRNA expression and BDNF secretion by genetically engineered fibroblasts	80
Effects of striatal MPP+ infusion and BDNF-secreting fibroblasts on SNc	80
Effects of BDNF-secreting fibroblasts on SN DA levels and metabolism	83
Discussion	86
CHAPTER IV: Antioxidative mechanism of neurotrophic factor-mediated neuroprotection against mitochondrial toxicity	91
Abstract	91
Introduction	92
Materials and methods	96
Cell preparation, surgical implantation, and 3-NP lesion procedures	96
Histological analysis	96
Neurochemical analysis	97
Statistical analysis	98
Results	98
Discussion	103

Conclusion	105
CHAPTER V: Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology	107
Abstract	107
Introduction	108
Materials and methods	112
Lesion surgery, behavioral testing, and experimental groups	112
Preparation and transplantation of porcine fetal ventral mesencephalon	115
Perfusion and histological processing	116
Morphometric and statistical analyses	118
Results	119
Effects of porcine ventral mesencephalic grafts on rotational asymmetry	119
Survival and size of porcine ventral mesencephalic grafts	122
Correlation between TH+ neuron survival and functional recovery	125
Morphology and organization of fetal porcine ventral mesencephalic grafts	125
Discussion	135
CHAPTER VI: General discussion	142
Therapeutic approaches to the treatment of Parkinson's disease	142
Limitations of current therapies for Parkinson's disease	142
Alternative approaches to the treatment of Parkinson's disease	144
Experimental approaches to neuroprotection of dopaminergic neurons	144
Neuroprotection in a rat model of Parkinson's disease	144
Mechanism of neuroprotection in a rat model of mitochondrial impairment	148
Considerations for clinical use of neurotrophic factors	149
Experimental approaches to neurotransplantation of dopaminergic neurons	152
Transplantation of fetal porcine dopaminergic neurons	152

Properties of fetal porcine grafts	153
Xenotransplantation and immunoprotective strategies	154
Strategies to increase neuronal survival	156
Considerations in clinical neurotransplantation	158
Conclusions	159
REFERENCES	162

FIGURES

Figure 1.	Basal ganglia circuitry and the role of dopamine	8
Figure 2.	Mechanisms of cellular injury and neurotrophic factor protection	21
Figure 3.	Neurotrophic factors and their receptors	26
Figure 4.	Engineering of BDNF-secreting fibroblasts for transplantation	52
Figure 5.	Schematic diagram of experimental paradigm	56
Figure 6.	Model of SNc degeneration after striatal MPP+ infusion	60
Figure 7.	Effects of BDNF-secreting fibroblast implants on dopaminergic cell degeneration in the SNc	64
Figure 8.	Regression analysis graph of striatal lesion maximal cross-sectional area versus cell survival in BDNF[-] and BDNF[+] implanted animals	66
Figure 9.	Schematic and histological representation of BDNF-mediated neuroprotection	78
Figure 10.	BDNF mRNA expression and BDNF secretion by genetically engineered fibroblasts	82
Figure 11.	Effects of BDNF-secreting fibroblasts on SN DA levels and metabolism	85
Figure 12.	Formation of peroxynitrite	95
Figure 13.	Effect of 3-NP on DARPP-32 striatal immunoreactivity	100
Figure 14.	Effect of NGF-secreting fibroblasts graft on 3-NP induced generation of 3-nitrotyrosine <i>in vivo</i>	102

Figure 15.	Schematic diagram of transplantation and behavioral testing methods	114
Figure 16.	Amphetamine-induced net rotational asymmetry	121
Figure 17.	TH+ neuronal survival and graft volume of intrastriatal porcine xenografts	124
Figure 18.	Relationship between TH+ neuron survival and extent of functional recovery	127
Figure 19.	In situ hybridization for PRE DNA in intrastriatal porcine VM graft	129
Figure 20.	TH immunostained VM graft	132
Figure 21.	Histological analysis of porcine VM xenograft of CyA treated and non-CyA treated rat	134

Abbreviations

BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
CD44	Cluster of differentiation antigen 44
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CRL	Crown-to-rump length
CyA	Cyclosporine A
DA	Dopamine
DARPP-32	Dopamine- and c-AMP-regulated phosphoprotein 32 KDa
DOPAC	3,4 dihydroxyphenylacetic acid
DRG	Dorsal root ganglion
ED	Embryonic day
EIA	Enzyme-linked immunoassay
GDNF	Glial cell line-derived neurotrophic factor
GPe	Globus pallidus, external segment
GPi	Globus pallidus, internal segment
HBSS	Hanks balanced salt solution
HD	Huntington's disease
IL-2	Interleukin-2
L-dopa	Levodopa
MAO-B	Monoamine oxidase-B
MFB	Medial forebrain bundle

MHC	Major histocompatibility complex
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NBS	Normal blocking serum
NGF	Nerve growth factor
NF-70	Neurofilament 70,000 mol. wt
NMDA	N-methyl-D-aspartate
NOS	Nitric oxide synthase
3-NP	3-Nitropropionic acid
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
NTF	Neurotrophic factor
OD	Optical density
6-OHDA	6-Hydroxydopamine
PBS	Phosphate buffered saline
PD	Parkinson's disease
PET	Positron emission tomography
PRE	Porcine repeat element
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SOD	Superoxide dismutase
SSC	Sodium chloride-sodium citrate
STN	Subthalamic nucleus
TBS	Tris-buffered saline

TH	Tyrosine hydroxylase
VM	Ventral mesencephalon
VTA	Ventral tegmental area

CHAPTER I

INTRODUCTION

Parkinson's disease

Clinicopathological features. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by bradykinesia, rigidity, resting tremor, and postural instability (Hoehn and Yahr, 1967). Parkinsonian patients characteristically have a stooped posture, walk with a shuffling gait, and show reduced facial expression. In addition to the classical clinical symptomatology in PD, there are pathognomonic neuropathologic changes defined by diffuse distribution of cytoplasmic Lewy bodies and cell loss within the substantia nigra (SN) (Fearnley and Lees, 1994; Marsden, 1982a). In addition, pathologic features may also be observed in the locus coeruleus, dorsal motor vagal nucleus, nucleus basalis of Meynert, and cortex. Dysfunction of the nigrostriatal system associated with dopaminergic cell loss in the substantia nigra pars compacta (SNc) and the secondary depletion of striatal dopamine (DA) levels likely account for the motor deficits of PD (Marsden, 1982b). The degree of neuronal loss in the SNc is paralleled by the reduction of striatal DA (Bernheimer et al., 1973), with levels in the putamen more severely depleted than the caudate (Kish et al., 1988; Nyberg et al., 1983). Moreover, there is a rostrocaudal gradient of DA depletion in which the caudal putamen is the most affected (Kish et al., 1988).

As a consequence of the striatal DA depletion, presynaptic and postsynaptic compensatory changes occur in the PD brain. DA turnover in the remaining DA neurons increases (Bernheimer et al., 1973) and receptor supersensitivity develops (Lee et al., 1978). Thus, while the loss of striatal DA correlates with the severity of clinical disability in PD, clinical manifestations are not apparent until 80-85% of nigral neurons have degenerated and striatal DA levels are depleted by 80% (Marsden, 1982a). Since pathologic changes precede the manifestation of clinical symptoms, it is of interest to develop strategies to protect remaining DA neurons during this subclinical stage in order to prevent the progression of cell loss and onset of clinical dysfunction.

Epidemiology. Most cases of parkinsonism are due to degenerative processes and are classified as idiopathic PD. However, parkinsonian signs may also be associated with multisystem atrophy, cerebrovascular disease, postencephalitic parkinsonism, toxin ingestion (e.g., manganese, see MPTP below) or drug intoxication (e.g., neuroleptics). Parkinsonism is rarely attributable to tumor or trauma (Jellinger, 1986).

Estimates for the prevalence of PD range from approximately 100 - 300 cases per 100,000 individuals (0.1%-0.3%); and this rate increases with increasing age (Marttila and Rinne, 1981; Rajput, 1992). Males and females show similar prevalence rates, and whites are more commonly affected than are other races (Tanner, 1992). While it is unclear to what extent environmental factors may cause PD, exposure to well water, rural environments, farming, and herbicides has been associated with an increased risk for developing PD (see Tanner, 1992 for review). In addition, cigarette smoking had been found to be inversely

correlated with PD, suggesting a possible preventative effect (Baumann et al., 1980; Godwin-Austen et al., 1982; Marttila and Rinne, 1980). No relationship, however, has been found between the degree of smoking and the severity of PD (Golbe et al., 1986). Interestingly, it has recently been reported that monoamine oxidase-B (MAO-B) levels are reduced in the brains of smokers, and it has been suggested that decreased production of reactive oxygen species due to lower levels of MAO may account for the suggested neuroprotective effect (Fowler et al., 1996).

Etiology and Pathogenesis. The cause of DA neuron loss in the SNc in idiopathic PD is not known. Based on low concordance rates amongst monozygotic twins, a significant mendelian genetic component to the etiology of idiopathic PD was initially considered unlikely (Duvoisin, 1986; Marsden, 1987; Marttila et al., 1988; Ward et al., 1983). However, reevaluation of the twin studies (Golbe, 1990) as well as assessment of additional families (Maraganore et al., 1991; Vieregge et al., 1992) suggest there is an increased familial tendency for PD, with an approximate 3.5-fold greater risk for PD in first degree relatives of an affected individual compared to relatives of control subjects (Payami et al., 1994). Furthermore, positron emission tomography (PET) scan studies have revealed a significant decrease in fluorodopa uptake in asymptomatic co-twins, with a concordance rate of 45% in monozygotic twins (Burn et al., 1992).

There are also some kindreds which display autosomal dominant parkinsonism (e.g., Golbe et al., 1990; Markopoulou et al., 1995; Waters and Miller, 1994; Wszolek et al., 1995). Yet, in some of these cases, earlier onset (Markopoulou et al., 1995; Waters and Miller, 1994) and a more aggressive

clinical course (Golbe et al., 1990) as well as atypical pathology have been observed (Waters and Miller, 1994). Genetic analyses in families with autosomal dominant parkinsonism have failed thus far to identify a genetic cause for PD (Bandmann et al., 1995; Gasser et al., 1994; Parboosingh et al., 1995), and no association with a CAG trinucleotide repeat has been found (Carrero-Valenzuela et al., 1995) as has been shown for other neurodegenerative disorders such as Huntington's disease (HD) (The Huntington's Disease Collaborative Research Group, 1993).

The development of parkinsonism following the administration of the mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, see below) (Langston et al., 1983) suggests that a structurally similar environmental or endogenous agent may cause PD by a similar mechanism of neurotoxicity. However, no such environmental risk factor has been identified (Tanner, 1989). The precise role of heredity and environment remains unclear, but it has been suggested PD may be due to an interplay of toxin exposure, genetic susceptibility, and aging. Cell loss due to a genetic susceptibility to a toxic insult, in combination with the progressive loss of nigrostriatal neurons associated with normal aging (McGeer et al., 1977; Scherman et al., 1989), may surpass a threshold of DA cell loss beyond which symptomatic PD ensues (Calne and Langston, 1983).

Clinical abnormalities and post-mortem findings in PD patients have led to various hypotheses regarding the mechanisms for progressive cell loss in PD. Several lines of evidence suggest that oxidative stress contributes to the pathogenesis of PD. Post-mortem analyses have demonstrated increased lipid peroxidation in the SN of PD brains suggesting either an excess production of

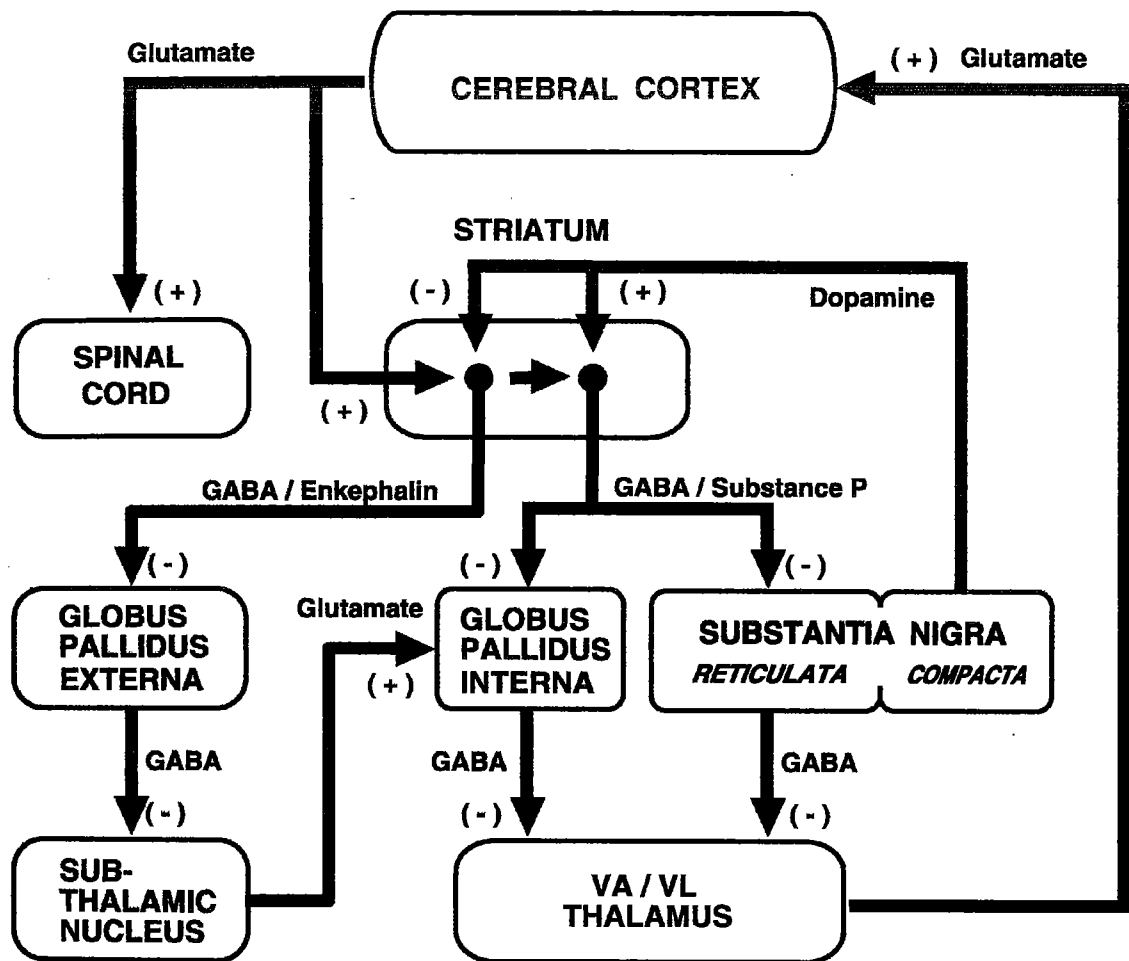
neurotoxic free radicals or a failure of the normal protective mechanisms (Dexter et al., 1986; Dexter et al., 1989; Dexter et al., 1994). Several detoxifying systems have been found to be altered in the PD brain including decreased activities of catalase, peroxidase (Ambani et al., 1975), and glutathione peroxidase (Kish et al., 1985) as well as decreased levels of reduced glutathione (Perry et al., 1982; Sian et al., 1994a). The decreased levels of glutathione are likely due to in part to increased breakdown as the degradative enzyme γ -glutamyltranspeptidase, has been found to be elevated in parkinsonian brains (Sian et al., 1994b). In addition, elevated iron levels are found in the SN of PD patients (Dexter et al., 1987). Since iron can generate cytotoxic free radicals, high levels of iron may contribute to oxidative stress and lipid peroxidation. The dopaminergic neurons of the SN may be particularly vulnerable to oxidative stress due to the metabolism of DA (Olanow, 1990). DA is oxidized to hydrogen peroxide via MAO-B. In the presence of iron, hydrogen peroxide is metabolized to reactive hydroxyl radicals via the Fenton reaction. Thus, the elevated iron levels found in the PD brain, combined with impaired antioxidant mechanisms may exacerbate ongoing free radical generation and oxidative damage (Fahn and Cohen, 1992; Olanow, 1990). Further post-mortem as well as clinical studies have demonstrated mitochondrial abnormalities in PD patients. Similar to the inhibition of mitochondrial function produced by MPTP, defects have been found in complex I of the electron transport chain in SN (Schapira et al., 1990; Schapira et al., 1989), platelet (Haas et al., 1995; Parker et al., 1989), and muscle (Shoffner et al., 1991) mitochondria preparations from PD subjects. Such findings suggest PD may be due to an inherited mitochondrial defect. However, no known mitochondrial genetic defect or deletion has been detected (Shoffner et al., 1991). The significance of

these findings with respect to the etiology of PD remains to be determined as it is unclear if these abnormalities cause neuronal degeneration or are a consequence thereof.

Functional organization of the basal ganglia and the role of dopamine.

The clinical manifestations of PD can be understood by examining the role of DA in the basal ganglia (Figure 1). The basal ganglia motor circuit is comprised of two parallel pathways which process cortical glutamatergic input (Albin et al., 1989; Alexander and Crutcher, 1990; DeLong, 1990). The direct pathway refers to striatal GABAergic and substance P inhibitory fibers which project to the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). In turn, GABAergic fibers from the SNr and GPi project to the thalamus, which sends excitatory fibers to the cortex. The indirect pathway projects to the GPi and SNr via a more circuitous route: Striatal GABAergic and enkephalin containing fibers project from the striatum to the external segment of the globus pallidus (GPe), and GABAergic fibers from the GPe project to the subthalamic nucleus (STN). The STN sends excitatory fibers to the GPi and SNr as well as to the GPe and cortex. Thus, activation of the direct pathway facilitates thalamocortical transmission whereas activation of the indirect pathway inhibits such activity. The nigrostriatal DA neurons serve to modulate this circuit by inhibiting the indirect and facilitating the direct pathway. These effects are likely mediated by the differential expression of DA receptor subtypes on striatal projection neurons. D1 receptors are expressed by neurons of the direct pathway, whereas D2 receptors are expressed by neurons of the indirect pathway (Robertson, 1992). Thus, in PD, where DA levels are depleted, the indirect

Figure 1. Basal ganglia circuitry and the role of DA. DA normally facilitates the direct pathway and inhibits the indirect pathway. In PD, the direct pathway is inhibited whereas the indirect pathway is active due to the loss of DA.



pathway is active whereas the direct pathway is inhibited, thereby producing thalamocortical inhibition (DeLong, 1990).

Pharmacological treatment strategies. The mainstay therapy for PD remains pharmacological replacement of DA via the oral administration of levodopa (L-dopa), the precursor to DA (Calne, 1993). L-dopa is absorbed in the proximal intestine, crosses the blood-brain-barrier (BBB), and is converted to DA via aromatic L-amino acid decarboxylase (dopa decarboxylase) contained in the nerve terminals of dopaminergic as well as non-dopaminergic neurons of the striatum (Hefti and Melamed, 1980; Hornykiewicz, 1974). As most L-dopa is metabolized prior to reaching the brain, co-administration of a peripheral dopa decarboxylase inhibitor such as carbidopa improves the efficacy and decreases the peripheral side effects of this drug (Cotzias et al., 1969; Koller and Hubble, 1990). L-dopa is highly effective in reducing bradykinesia and rigidity, yet tremor and postural instability are less improved (see Koller and Hubble, 1990 for review). While L-dopa therapy offers dramatic clinical improvement, long-term therapy is associated with significant side-effects including dyskinesias and psychosis as well as altered drug responses characterized as "end-of-dose deterioration" or "on-off phenomena" (Fahn, 1974; Marsden, 1982a; Marsden and Parkes, 1976). The precise physiologic processes underlying these effects are unclear, but they are thought to be associated with the fluctuations in L-dopa levels as well as DA receptor alterations (Chase et al., 1993). Indeed, motor fluctuations decline during continuous steady-state infusions of L-dopa suggesting that the intermittent dosing of L-dopa contributes to the observed motor abnormalities (Mouradian et al., 1989). As these motor fluctuations may

be associated with the duration of L-dopa therapy, treatment with L-dopa is usually delayed until function is impaired (Melamed, 1986; Yahr, 1977). Moreover, it has been hypothesized that L-dopa itself may be deleterious to dopaminergic neurons. The metabolism of DA can generate free radicals (Olanow, 1990), and DA has been shown to be toxic for dopaminergic neurons in culture (Michel and Hefti, 1990). Furthermore, increased DA turnover is associated with the generation of hydrogen peroxide (Spina and Cohen, 1989), suggesting that L-dopa administration to the PD brain may similarly cause oxidative stress (Olanow, 1990). Indeed, L-dopa treatment reduces brain mitochondrial complex I activity *in vitro* and *in vivo* (Przedborski et al., 1993).

In addition to L-dopa, direct DA agonists (e.g., bromocriptine and pergolide) or anticholinergic drugs, which counter the acetylcholine-DA imbalance, are useful alternatives or adjuncts to L-dopa therapy (Calne, 1993). Recent treatment strategies have also included the initiation of the MAO-B inhibitor deprenyl (selegiline) soon after the onset of parkinsonian signs (Clough, 1991). The rationale for deprenyl use in PD is based on the experimental finding that MPTP-induced parkinsonism in animal models can be prevented by inhibiting MAO-B (Heikkila et al., 1984b; Langston et al., 1984). Furthermore, as DA metabolism is associated with the generation of free radicals via the Fenton reaction, inhibition of MAO-B catalyzed DA metabolism may decrease oxidative stress in the SN (Olanow, 1990). Initial clinical studies indicated that deprenyl administration delays the onset of symptoms requiring L-dopa therapy, suggesting this agent may retard disease progression (Parkinson Study Group, 1989; Tetud and Langston, 1989). More recent follow-up studies, however, have concluded that the beneficial effects of deprenyl are likely symptomatic, due

merely to attenuation of symptoms attributable to elevated levels of DA rather than to neuroprotection (Parkinson Study Group, 1993; Parkinson Study Group, 1996b; Schulzer et al., 1992). Additionally, prior treatment with deprenyl has been shown to have no effect on the development of L-dopa associated complications (Parkinson Study Group, 1996a). Parallel studies evaluating the ability of the anti-oxidant tocopherol, the active component of vitamin E, to delay the need for L-dopa treatment have failed to demonstrate any benefit (Parkinson Study Group, 1993; Parkinson Study Group, 1996a; Parkinson Study Group, 1996b).

While pharmacological replacement therapy with L-dopa initially provides significant symptomatic relief, decreased effectiveness and adverse effects are associated with long-term treatment (Marsden, 1982a). The limitations of present pharmacological treatments are clear and indicate the need for alternative DA replacement and neuroprotective therapies.

Surgical treatment strategies.

Functional neurosurgery. Historically, the treatment for PD involved surgical lesioning of the corticospinal tracts and subsequently the stereotactic lesioning of the extrapyramidal motor system, specifically the thalamus (thalamotomy) or pallidum (pallidotomy) (see Goetz et al., 1993; Hauser et al., 1995 for review). With the advent of pharmacological therapies such as L-dopa, surgical treatment declined. However, the untoward effects of long-term L-dopa therapy, coupled with a greater understanding of neuronal circuitry of the basal ganglia and the advances of stereotactic neurosurgery, have resulted in a resurgence of surgical approaches for the treatment of PD.

The rationale for lesioning is to interrupt the basal ganglia neuronal circuitry, thereby preventing the activation of the indirect pathway and reestablishing the activity of the direct pathway which are altered in the parkinsonian brain due to the DA deficiency. Thalamotomy has been shown to be of benefit to patients with refractory tremor (Tasker, 1990). Rigidity may also be improved by this procedure, yet bradykinesia is not affected. Similarly, high frequency electrical stimulation in the thalamus is also associated with a reduction in tremor, and this approach permits bilateral treatment (Benabid et al., 1991). Lesions of the posteroventral pallidum have been reported to improve tremor, rigidity, and bradykinesia as well as L-dopa associated dyskinesias (Laitinen, 1995; Laitinen et al., 1992). Possible complications associated with thalamotomy include transient paresis, cognitive disorders, parasthesia, dysarthria, and gait disturbances (Tasker, 1990), whereas the primary complication associated with pallidotomy is visual field defects due to the proximity of the lesion site to the optic tract (Laitinen et al., 1992). While the present surgical results are encouraging, it remains to be determined if the beneficial effects are long-lasting and what long-term sequelae may be associated with ablative lesioning of the brain.

Neurotransplantation. An alternative to pharmacologic replacement therapy is physiologic replacement of DA by transplanting DA-releasing cells. Early clinical transplantation studies involved the implantation of catecholamine-producing cells from the adrenal medulla into the DA-depleted striatum of PD patients (Goetz et al., 1989; Lindvall et al., 1987; Madrazo et al., 1987; see Freed et al., 1990b for review). While the aim of these studies was to provide an autologous cell source of DA production, results were highly variable and of no

sustained therapeutic benefit. Due to poor graft survival and the high rates of morbidity and mortality associated with this approach, adrenal medullary transplantation has been abandoned (Ahlskog, 1993; Goetz et al., 1991).

Based on transplantation studies conducted in rodent and primate models of PD (see below), clinical trials were initiated to evaluate the efficacy of intrastriatal transplantation of human fetal ventral mesencephalon (VM) cell suspensions to patients with PD. Initial studies in two patients with idiopathic PD showed only marginal clinical effects (Lindvall et al., 1990b; Lindvall et al., 1989). Using a modified protocol with decreased tissue storage time and a thinner implantation cannula, encouraging results were found with the subsequent two idiopathic PD cases (Lindvall et al., 1990a; Lindvall et al., 1992; Sawle et al., 1992). Unilateral grafts of fetal human VM into the putamen decreased rigidity and bradykinesia, predominantly on the side contralateral to the grafts, and also decreased the number of daily "off" periods and time spent "off" (Lindvall et al., 1990a; Lindvall et al., 1992). Clinical improvement was apparent at approximately 6-12 weeks post-transplantation, and the degree of recovery correlated with graft survival and function as evaluated by 18F-fluorodopa uptake using PET at one year post-transplantation (Lindvall et al., 1990a; Lindvall et al., 1992; Sawle et al., 1992). In a follow-up study, long-term graft survival was assessed in these two patients at three years post-transplantation (Lindvall et al., 1994). Both patients showed near normal 18F-fluorodopa uptake in the grafted regions. In one patient, 18F-fluorodopa uptake by the graft remained unchanged from that measured one year post-operative while nongrafted striatal sites showed decreased uptake indicative of progressive degeneration associated with the underlying disease process. The other patient

showed a continued increase in uptake and was able to discontinue L-dopa medication. Transplantation of two additional patients with idiopathic PD using an identical protocol has shown a similar course of clinical improvement as well as positive PET scan results (Peschanski et al., 1994). Furthermore, bilateral cell suspension grafts in the caudate and putamen of two patients with MPTP-induced parkinsonism have produced marked clinical improvement and a corresponding increase in striatal uptake of fluorodopa (Widner et al., 1992).

Additional studies have been undertaken implanting solid pieces of tissue, rather than dissociated cell suspensions, into the striatum of PD patients (Freed et al., 1992; Freed et al., 1990a; Freeman et al., 1995; Kordower et al., 1995). Sustained clinical improvement accompanied by increased fluorodopa uptake has been reported up to 46 months following transplantation in one patient and variable degrees of improvement have been observed in other patients of this series (Freed et al., 1992). Likewise, in another series of four patients receiving bilateral transplantation of solid tissue into the putamen, a significant degree of recovery and a parallel increase in fluorodopa uptake was apparent at 6 months after transplant (Freeman et al., 1995). A single patient from this group died 18 months after grafting due to a pulmonary embolus, permitting the neuropathological analysis of the graft (Kordower et al., 1995). This patient showed functional recovery and a progressive increase in fluorodopa uptake, and histological analysis revealed surviving grafts which were well integrated with the host striatum. Extensive tyrosine hydroxylase (TH) neuronal survival and fiber outgrowth were apparent indicating that grafted fetal cells can survive long-term in the parkinsonian brain and reduce symptoms.

Results thus far have demonstrated the efficacy and potential utility of transplantation as a therapeutic modality for the treatment of PD. However, several variables including cell preparation and graft location as well as number of cells need to be refined and optimized before neuronal transplantation can be viewed as a therapeutic alternative for PD patients. Moreover, since transplantation does not halt the progressive degeneration of PD, and since this progression may counteract the graft-induced recovery, protective therapies remain of interest.

Animal models of Parkinson's disease

The development of animal models of PD have facilitated the investigation of neurotransplantation and neuroprotection of dopaminergic neurons. In addition, these models have contributed to the understanding of possible etiologies of parkinsonism. While no animal model fully parallels the progressive neuronal death seen in PD, the administration of toxins produces useful models of dopaminergic neuronal degeneration. The models most commonly employed involve the administration of the neurotoxins 6-hydroxydopamine (6-OHDA) or MPTP.

6-Hydroxydopamine. Intracerebral injection of the neurotoxin 6-OHDA results in selective uptake by catecholamine membrane transporters and the irreversible destruction of dopaminergic and noradrenergic neurons (Ungerstedt, 1968). The precise mechanism for this neurotoxicity remains unclear but likely

involves the formation of H_2O_2 and free radicals by auto-oxidation or the formation of products which act as alkylating agents (Cohen and Heikkila, 1974; Heikkila and Cohen, 1971; see Jonsson, 1980 for review).

Unilateral lesioning of the nigrostriatal pathway with 6-OHDA provides a useful behavioral paradigm for the study of PD, and in particular the extent of dopaminergic reinnervation following transplantation. In this lesion model, motor asymmetry is apparent due to striatal DA imbalance (Ungerstedt, 1968). Administration of amphetamine causes DA release from the intact nigrostriatal system and results in an excess of DA in the intact striatum relative to the lesioned side. As a consequence, unilaterally lesioned rats administered amphetamine will rotate toward the lesioned side (ipsilateral rotation) thereby providing a quantifiable measure of striatal DA innervation (Ungerstedt, 1971c; Ungerstedt and Arbuthnott, 1970). Rats which rotate at least 7 turns per minute have been found to have 97-99% striatal DA depletion (Schmidt et al., 1983; Schmidt et al., 1982). In addition, 6-OHDA induced striatal denervation is associated with receptor supersensitivity (Ungerstedt, 1971b). Administration of the DA receptor agonist apomorphine thus results in greater activation of the lesioned side relative to the intact striatum and consequently rotation away from the lesioned side (contralateral rotation) (Marshall and Ungerstedt, 1977; Ungerstedt, 1971b). Furthermore, this lesion model produces sensorimotor and paw-reaching deficits in rats (Ljungberg and Ungerstedt, 1976; Whishaw et al., 1986). Bilateral injections of 6-OHDA produce akinesia as well as adipsia and aphagia thus precluding the use of the bilateral lesion model for most transplantation studies (Ungerstedt, 1971a).

The behavioral deficits associated with unilateral 6-OHDA lesions and the improvement following neuronal transplantation demonstrate the utility of this model for evaluating striatal innervation and functional recovery associated with neuronal transplantation (see below). However, while the 6-OHDA lesion model parallels the neurochemical depletion characteristic of PD, it does not lend itself to studies on the etiology of PD as 6-OHDA is highly toxic and acts by an incompletely understood mechanism.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is a meperidine analog which produces irreversible parkinsonian symptoms in humans (Langston et al., 1983) and has been used extensively to produce experimental models of PD in animals (Burns et al., 1983; Heikkila et al., 1984a). MPTP is converted to 1-methyl-4-phenylpyridinium (MPP⁺), the active metabolite which mediates neurotoxicity, in a reaction catalyzed by MAO-B. Inhibition of this enzyme prevents both the conversion of MPTP to MPP⁺ and hence the neurotoxicity associated with MPTP administration (Heikkila et al., 1984b; Langston et al., 1984). The conversion of MPTP to MPP⁺ is localized to serotonergic neurons and astrocytes, paralleling the distribution of MAO-B in brain (Levitt et al., 1982). MPTP administration results in loss of DA cells in the SNc and depletion of DA and its metabolites in the neostriatum (Burns et al., 1983; Heikkila et al., 1984a; Langston et al., 1983). In addition, serotonergic and noradrenergic neurons as well as glia may be affected (Burns et al., 1983; Heikkila et al., 1985; Ter Horst et al., 1992).

The pattern of neurotoxicity produced by MPTP is accounted for by its mechanism of action. MPTP preferentially binds to sites in the striatum, SN, and

locus coeruleus where it is converted to MPP⁺ by MAO-B (Javitch et al., 1984). MPP⁺ is subsequently taken up by catecholaminergic transporters at noradrenergic and DA nerve terminals (Javitch et al., 1985). As rats lack MAO-B in the nigrostriatal pathway, the active metabolite MPP⁺ must be administered intracranially in order to achieve dopaminergic neuronal loss modelling PD. In the striatum, MPP⁺ is retrogradely transported to the SNc thus accounting for the neuronal loss in the SNc and depletion of striatal DA (Campbell et al., 1990; Herkenham et al., 1991). The neurotoxicity of MPP⁺ is associated with the inhibition of mitochondrial oxidative phosphorylation (Nicklas et al., 1985) at complex-I of the electron transport chain (Nicklas et al., 1987). As a consequence, there is decreased ATP production (Chan et al., 1991). It has been suggested that this loss of ATP may result in excitotoxic cell death as decreased ATP is associated with membrane depolarization, release of magnesium blockade of the N-methyl-D-aspartate (NMDA) receptor, and subsequent receptor activation and excitotoxicity (Storey et al., 1992). The role for NMDA-receptor mediated excitotoxicity in the mechanism of MPP⁺ neurotoxicity is supported by the findings that inhibition of glutamatergic input by an antagonist or decortication is able to prevent MPP⁺ induced nigral degeneration (Srivastava et al., 1993).

The finding that the neurochemical, anatomical, and behavioral abnormalities of MPTP-induced parkinsonism resemble idiopathic PD suggests there may be a common underlying mechanism of neuronal degeneration. This feature demonstrates the utility of the MPTP model in developing experimental strategies to protect against neuronal degeneration in PD.

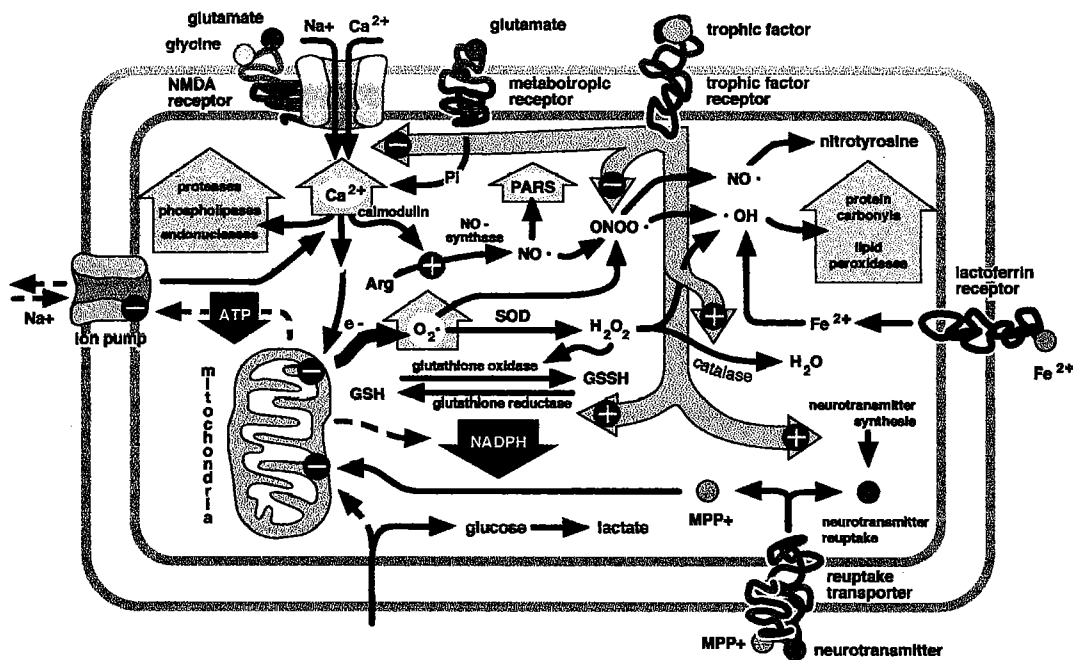
Neurotrophic factors

Mechanisms of cellular injury and neurotrophic factor interventions.

The pathogenesis of neurodegenerative diseases is likely mediated by impaired cellular energy metabolism coupled with oxidative stress and excitotoxicity (Albin and Greenamyre, 1992; Beal, 1992; Beal, 1995). Excitotoxic neuronal death is induced by glutamate receptor activation which results in calcium influx as well as activation of an enzymatic cascade which produces free radicals and further calcium overload (Figure 2). As a consequence of the increased intracellular calcium levels, cellular proteases such as calpain I and phospholipases are activated resulting in the degradation of structural proteins and loss of cell membrane integrity. Furthermore, oxygen radicals are generated due to the release and metabolism of arachadonic acid as well as the conversion of xanthine dehydrogenase to xanthine oxidase (see Choi, 1988; Choi, 1992 for review). Nitric oxide synthase (NOS) is also activated in response to increased intracellular calcium (Garthwaite et al., 1988), and inhibition of NOS attenuates NMDA-induced neurotoxicity, suggesting a role for nitric oxide in glutamate toxicity (Dawson et al., 1991c). Abnormal glutamate receptor activation alone may account for some of the neuronal death seen in neurodegenerative diseases. It is likely, however, that an interaction of cellular metabolic stress and oxidative damage can result in secondary excitotoxic cell death (see Beal, 1995 for review).

Neurotrophic factors (NTFs) are able to protect neurons against a variety of cellular insults indicating their potential therapeutic value for the treatment of neurodegenerative disorders (Mattson et al., 1993). Among the mechanisms

Figure 2. Mechanisms of cellular injury and neurotrophic factor protection. Neuronal death in degenerative diseases is mediated by excitotoxicity and metabolic dysfunction resulting in loss of calcium homeostasis and production of free radicals. Glutamate receptor activation results in calcium influx which is inadequately buffered when coupled with mitochondrial impairment (aging, MPP+, 3-NP). As a consequence, intracellular calcium levels rise and cause a cascade of enzyme activation and free radical production. Neurotrophic factors may protect against neurodegeneration by maintaining intracellular calcium or by increasing the activity of free radical scavengers such as catalase, superoxide dismutase (SOD), and glutathione reductase. (From Galpern et al., in press; modified from Beal, 1995, with permission)



underlying NTF protection are the maintenance of calcium homeostasis as well as the reduction in levels of free radicals by increasing antioxidant enzyme activities (Cheng and Mattson, 1991; Mattson et al., 1995). By decreasing cellular oxidative stress or by interfering in the cascade to cell death, NTFs may reduce neuronal vulnerability and protect against ensuing neurodegenerative processes.

Role of neurotrophic factors. NTFs are important determinants of neuronal development and organization, affecting innervation of target tissue as well as survival of neurons (Purves, 1986). Classically, NTFs are secreted during specific developmental stages by target tissues in limited quantities (Barde, 1988). While it was originally thought that different neuronal populations were each responsive to only a single NTF, there is overlap and redundancy whereby a single NTF may affect more than one cell type and a specific cell type may respond to several NTFs (Korsching, 1993). Moreover, actions of NTFs are associated not only with retrograde transport from the target tissue but also autocrine and paracrine mechanisms (Kokaia et al., 1993; Miranda et al., 1993). Similarly, NTF expression in the adult brain suggests various mechanisms of action in relation to the observed selective neuronal trophism. NTFs are important for neuronal maintenance in the adult brain, and insufficiency of such trophic support due to decreased NTF supply or impaired target cell response may account for some of the cell death in neurodegenerative diseases (Appel, 1981; Hefti, 1983).

Classes of neurotrophic factors and regional expression.

Neurotrophins. Nerve growth factor (NGF) (Levi-Montalcini, 1987), brain-derived neurotrophic factor (BDNF) (Barde et al., 1982; Leibrock et al., 1989), neurotrophin-3 (NT-3) (Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990; Maisonpierre et al., 1990; Rosenthal et al., 1990), and neurotrophin-4/5 (NT-4/5) (Berkemeier et al., 1991; Hallbook et al., 1991; Ip et al., 1992) are structurally related and comprise the neurotrophin gene family (Korsching, 1993).

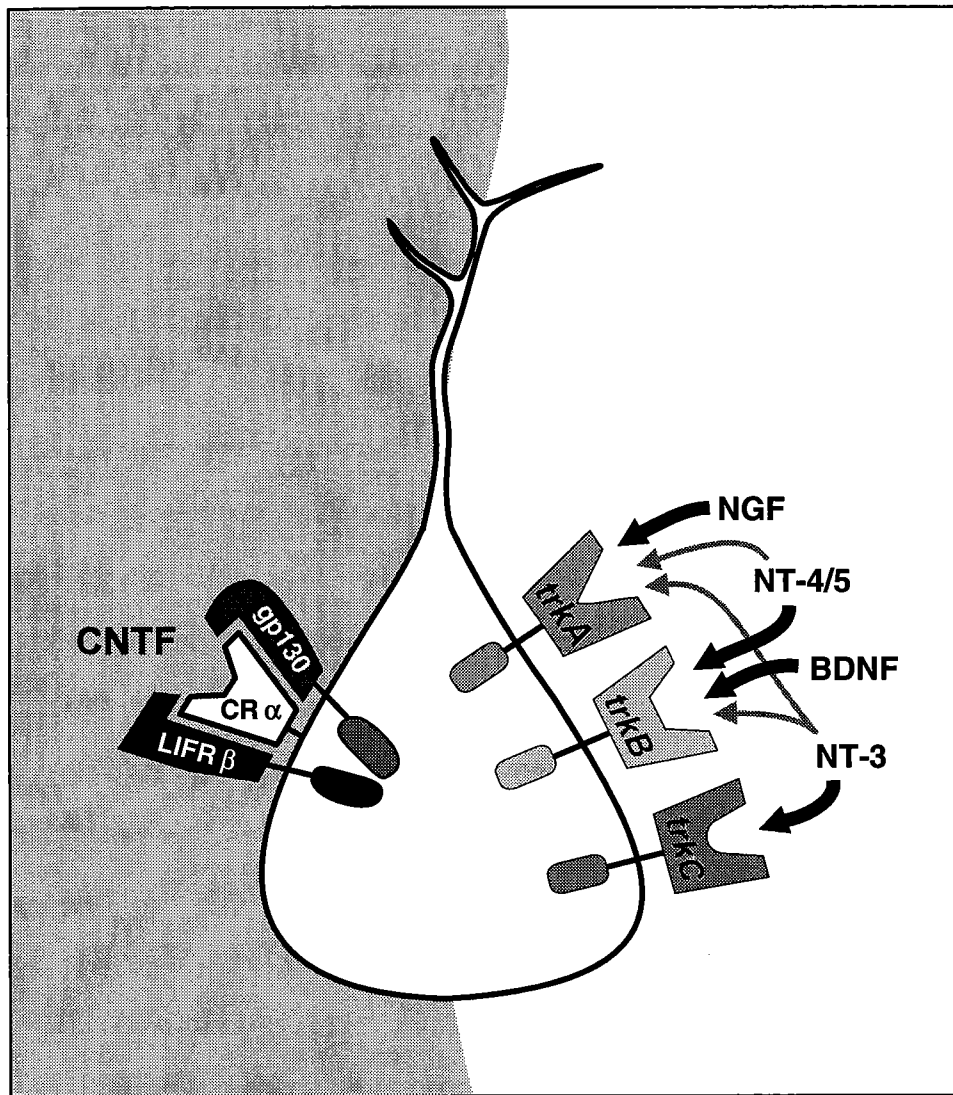
The highest levels of NGF are found in the neocortex and hippocampus of adult brain, the projection sites of basal forebrain cholinergic neurons, and lower levels are found within the striatum and other brain regions (Shelton and Reichardt, 1986; Whittemore et al., 1986). BDNF mRNA is distributed throughout the adult brain with the highest amounts localized to the hippocampus and cortex and lower amounts present in the striatum (Hofer et al., 1990; Wetmore et al., 1990) and DA neurons of the SNc (Gall et al., 1992; Seroogy et al., 1994). The level of BDNF mRNA expression in the hippocampus exceeds that of NGF (Hofer et al., 1990). NT-3 mRNA has been detected in the hippocampus, cerebellum and cortex of adult brain (Ernfors et al., 1990; Hohn et al., 1990; Jones and Reichardt, 1990) as well as DA neurons of the SNc (Gall et al., 1992; Seroogy et al., 1994). In the adult brain, NT-4/5 expression is highest in the pons-medulla, hypothalamus, and cerebellum, with lower expression found in the hippocampus, midbrain, striatum and septum (Timmusk et al., 1993).

Other neurotrophic factors. Ciliary neurotrophic factor (CNTF), a member of the alpha-helical cytokine superfamily, is a cytosolic factor (Lin et al., 1989; Stöckli et al., 1989). In the adult brain, CNTF mRNA is expressed at

moderate levels in the cerebellum and brainstem and at low levels in other brain regions including the hippocampus, striatum, cortex, and septum (Stöckli et al., 1991). Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor- β superfamily. GDNF mRNA is widely distributed in the adult CNS and has been localized to the striatum, hippocampus, cortex, VM, cerebellum, and spinal cord (Choi-Lundberg and Bohn, 1995; Schaar et al., 1993; Springer et al., 1994; Strömberg et al., 1993). Basic fibroblast growth factor (bFGF) is a mitogenic growth factor with neurotrophic activity within the CNS (Baird, 1994). bFGF mRNA is widely distributed throughout the brain in both astrocytes and neurons (Gonzalez et al., 1995). Platelet-derived growth factor (PDGF) is also a mitogen and occurs as three isoforms, PDGF-AA, PDGF-AB, and PDGF-BB. Transcripts for both PDGF chains A and B are found throughout the brain (Sasahara et al., 1991). Transforming growth factor- β (TGF- β) also occurs in several isoforms. TGF- β 2 and 3 mRNA is present in cortex, striatum, hippocampus, cerebellum, and brainstem (Unsicker et al., 1991).

Specificity and distribution of neurotrophic factor receptors in the adult brain. *Neurotrophins.* The biological actions of the neurotrophins are mediated by a family of receptor tyrosine kinases, Trks. These high affinity receptors contain an extracellular domain for ligand recognition, a transmembrane domain, and a cytoplasmic domain which possesses tyrosine kinase activity (Meakin and Shooter, 1992). These different receptors each show distinct and overlapping binding characteristics (Figure 3). The effects of NGF are mediated via the TrkA receptor (Kaplan et al., 1991a; Kaplan et al., 1991b; Klein et al., 1991a), whereas BDNF (Klein et al., 1991b; Soppet et al., 1991; Squinto et al., 1991)

Figure 3. Neurotrophic factors and their receptors. The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5) bind with high affinity to the tyrosine kinase receptors TrkA, TrkB, and TrkC. Relative affinities of each factor for the Trk receptors is represented by arrow thickness. The CNTF receptor complex is composed of the CNTF receptor ($CR\alpha$), leukemia inhibitory factor receptor (LIFR β), and gp130.



and NT-4/5 (Berkemeier et al., 1991; Ip et al., 1992; Klein et al., 1992) activate the TrkB receptor. NT-4/5 also binds with low affinity to the TrkA receptor (Berkemeier et al., 1991; Ip et al., 1992). NT-3 binds with high affinity to the TrkC receptor and with lower affinity to both TrkA and TrkB (Cordon-Cardo et al., 1991; Klein et al., 1991b; Lamballe et al., 1991; Soppet et al., 1991; Squinto et al., 1991). The neurotrophins bind not only to their respective Trk receptors, but also to the low affinity NGF receptor p75 (Meakin and Shooter, 1992). In addition to the full length TrkB receptor, there is a truncated form which lacks the cytoplasmic catalytic kinase domain (Klein et al., 1990; Middlemas et al., 1991). Similarly, different forms of the TrkC receptor exist, including a non-catalytic truncated variant (Tsoulfas et al., 1993; Valenzuela et al., 1993).

Trk receptors are expressed widely throughout the adult brain, predominantly by neurons. TrkA receptors are the least abundant and are expressed by the cholinergic interneurons of the striatum, neurons of the basal forebrain, as well as in various brainstem regions (Holtzman et al., 1995; Merlio et al., 1992; Richardson et al., 1986; Steininger et al., 1993). In situ hybridization studies indicate that there is similar regional expression of TrkB and TrkC mRNA (Altar et al., 1994c; Merlio et al., 1992). Transcripts for both receptors are found in numerous brain regions including the striatum, hippocampus, and cortex as well as SN and ventral tegmental area (VTA) (Altar et al., 1994c; Merlio et al., 1992). Additionally, the truncated form of TrkB is localized to the ependymal lining of ventricles, choroid plexus, and astrocytes (Altar et al., 1994c; Klein et al., 1990).

Other neurotrophic factors. The structure of the CNTF receptor is unrelated to the Trk receptor family and is comprised of three subunits, sharing

components with cytokine receptors for IL-6 and LIF (Davis et al., 1993; Davis et al., 1991; Davis and Yancopoulos, 1993). mRNA for the CNTF receptor is distributed throughout the brain, with highest expression in the cerebellum. High levels are also found in skeletal muscle (Davis et al., 1991). FGF receptor mRNA is widely expressed and is found at high levels in the hippocampus and at lower levels in the midbrain and several other brain regions (Wanaka et al., 1990). PDGF β receptors, which bind the PDGF-BB isoform, are found on neurons in the hippocampus, cerebellum, basal ganglia, and brainstem (Smits et al., 1991). The effects of TGF β are mediated by a high affinity serine-threonine kinase receptor (Massague, 1992). The receptor for GDNF is not yet known.

Dopaminergic neurotrophic factors. Due to the trophic effects of NTFs, the supplementation or replacement of a DA NTF may protect or slow the neuronal degeneration of PD. Several DA NTFs, including BDNF, NT-3, NT-4/5, bFGF, TGF β , and GDNF have recently been characterized (Hyman et al., 1991; Hyman et al., 1994; Hynes et al., 1994; Knusel et al., 1990; Lin et al., 1993; Mayer et al., 1993a; Poulson et al., 1994).

The effects of the neurotrophins BDNF (Hyman et al., 1991; Hyman et al., 1994; Knusel et al., 1991), NT-3 (Hyman et al., 1994), and NT-4/5 (Hyman et al., 1994; Hynes et al., 1994) as well as GDNF (Lin et al., 1993) on fetal DA neurons were first demonstrated *in vitro*. PDGF-BB (Nikkhah et al., 1993b), TGF β (Poulson et al., 1994) and bFGF also increases DA cell survival *in vitro*, yet the effect of bFGF is thought to be mediated by glia (Engele and Bohn, 1991; Knusel et al., 1990; Mayer et al., 1993a). In addition to promoting survival of DA neurons in culture, the administration of these factors to the intact adult rat brain

is associated with significant behavioral and neurochemical alterations. Supranigral delivery of BDNF enhances striatal DA turnover and decreases nigral DA turnover as well as causes contralateral rotations and locomotor activity in amphetamine treated rats (Altar et al., 1992; Martin-Iverson et al., 1994). The chronic administration of BDNF above the SN enhances the firing rate and number of electrically active DA neurons (Shen et al., 1994). The localization of mRNA for BDNF and its receptor, TrkB, to the SN in adult brain suggests that BDNF may maintain SNc neuronal function in the intact brain, perhaps in an autocrine or paracrine manner (Altar et al., 1994c; Gall et al., 1992; Hyman et al., 1994; Merlio et al., 1992; Seroogy et al., 1994). Also, exogenous BDNF delivered to the striatum can act on SNc DA neurons via receptor mediated retrograde transport (Mufson et al., 1994).

Similar to the *in vivo* effects of BDNF, supranigral infusion of NT-4/5 results in increased striatal DA turnover and release as well as contralateral rotation following the administration of amphetamine (Altar et al., 1994a), and NT-3 increases amphetamine-induced contralateral turning and decreases SN DA turnover (Martin-Iverson et al., 1994). Likewise, intranigral GDNF administration in intact adult rats increases spontaneous and amphetamine-induced locomotor behavior. These behavioral changes are associated with increased DA levels and turnover in the SN and increased DA turnover and decreased DA levels in the striatum (Hudson et al., 1995). In addition, sprouting of TH fibers near the injection site and increased striatal TH fiber staining were noted (Hudson et al., 1995). By injecting GDNF into the striatum, it has been demonstrated that this factor is retrogradely transported to the SN DA neurons thereby suggesting that GDNF may act as a target derived NTF (Tomic et al.,

1995b). These actions indicate that NTFs may be able to augment DA neuronal function in the adult brain.

Of relevance to the neurodegenerative processes of PD, pretreatment of DA neurons with BDNF protects against the neurotoxic effects of MPP⁺ and 6-OHDA *in vitro*, perhaps by increasing levels of the antioxidant enzyme glutathione reductase (Beck et al., 1992; Hyman et al., 1991; Spina et al., 1992). NT-4/5 (Hynes et al., 1994), bFGF (Park and Mytilineou, 1992), GDNF (Hou et al., 1996), and TGF- β (Kriegstein and Unsicker, 1994) also protect against the toxic effects of MPP⁺ *in vitro*.

Several DA NTFs can prevent cell death in models of neurodegeneration *in vivo*. BDNF has shown variable efficacy in protection in *in vivo* lesion paradigms. In rats with partial lesions of the nigrostriatal pathway induced by striatal infusion of 6-OHDA, concomitant supranigral administration of BDNF enhances striatal DA metabolism and reverses lesion induced rotational asymmetry (Altar et al., 1994b). However, BDNF or NT-3 did not alter SN DA levels nor protect against the loss of striatal DA nerve terminals in this partial lesion paradigm. Initial studies using BDNF failed to show protection of DA neurons in the SNc following axotomy of the medial forebrain bundle in rat (Knusel et al., 1992; Lapchak et al., 1993). However, another study suggests that BDNF is neuroprotective in the axotomy paradigm (Hagg, 1994). Also, intrastriatal grafts of BDNF-secreting fibroblasts have been shown to prevent DA neuronal degeneration associated with intrastriatal administration of 6-OHDA (Levivier et al., 1995).

GDNF is able to protect against SN neuronal degeneration in MPTP treated mice (Tomic et al., 1995a) as well as in rats following axotomy of the

medial forebrain bundle (Beck et al., 1995) or striatal 6-OHDA administration (Kearns and Gash, 1995; Sauer et al., 1995a). Interestingly, GDNF was shown not only to be neuroprotective, but also regenerative since administration following MPTP administration resulted in regeneration of TH fibers (Tomac et al., 1995a). Additionally, GDNF administration to the SN 4 weeks after partial 6-OHDA lesioning of the MFB decreased apomorphine-induced rotational asymmetry, increased SN DA and DOPAC content, and spared 10% of the SN DA neurons (Bowenkamp et al., 1995; Hoffer et al., 1994). Additional *in vivo* studies have demonstrated that NT-4/5 prevents 6-OHDA denervation-induced changes in striatal neurotransmitter gene expression (Sauer et al., 1995b). Continuous infusion of CNTF near the SN has been shown to prevent DA neuronal degeneration in the SNc following transection of the nigrostriatal pathway in adult rat (Hagg and Varon, 1993). However, in contrast to the aforementioned protection by GDNF and BDNF, TH expression was only slightly preserved.

Transplantation of fetal dopaminergic neurons

The rationale for transplanting fetal VM neurons into the parkinsonian brain is to replenish striatal DA levels and reconstruct the synaptic circuitry within the striatum. Significant information regarding striatal reinnervation and graft-mediated functional recovery has been attained from animal studies, leading to the initiation and development of fetal cell transplantation as a therapy for PD.

Initial transplantation experiments. Following the demonstration that grafts of fetal neurons are able to survive within the adult brain and innervate the host (e.g., Björklund et al., 1976; Stenevi et al., 1976), subsequent studies addressed the capacity of grafts derived from the fetal precursor of the SN, the VM, to produce functional recovery in the 6-OHDA rat model of PD. Graft-induced behavioral recovery was initially demonstrated following the transplantation of solid pieces of fetal VM into the ventricle (Perlow et al., 1979) or into a cortical cavity above the striatum (Björklund and Stenevi, 1979) of 6-OHDA lesioned rats. Methodological adaptations showed that cell suspensions of fetal VM tissue grafted directly into brain parenchyma show improved graft survival relative to solid tissue grafts and also produced rotational recovery (Björklund et al., 1980b; Schmidt et al., 1981). Use of the cell suspension technique permits implantation with minimal tissue damage into deeper brain structures than does the grafting of solid tissue pieces. Moreover, it is possible to determine the number and viability of cells prior to implantation and to graft at multiple sites (Björklund et al., 1983a; Björklund et al., 1983b).

Important variables in cell transplantation.

Donor age. Appropriate donor age is critical to achieve adequate graft survival (Björklund et al., 1980b; Schmidt et al., 1981). For cell suspension grafting, the optimal donor age for transplantation coincides with the developmental period in which the dopaminergic neurons of the SN differentiate and undergo their last cell division (Björklund et al., 1983a). For rat, this time point corresponds to embryonic day (ED) 14-15 at which point the crown-to-rump length (CRL) of the fetuses is approximately 10-14 mm (Björklund et al.,

1983a; Brundin et al., 1985b), and for human fetal tissue the optimal donor age is at 6.5 - 9 weeks post-conception (Brundin et al., 1986). Use of older tissue results in significantly reduced graft survival (Björklund et al., 1980b; Björklund et al., 1983a; Brundin et al., 1988a; Brundin et al., 1985b). However, donor age is less constrained when grafting solid tissue (Björklund et al., 1980a). Yet, solid tissue may be more immunogenic as vasculature will be at least partially donor-derived (Broadwell et al., 1987).

Implantation site: The location of the graft site and subsequent region of striatal reinnervation is important for the restoration of function. There is a topographic organization in the striatum which is reflected by the finding that grafts reinnervating the dorsal striatum produce rotational recovery (Björklund et al., 1980a; Dunnett et al., 1983; Dunnett et al., 1981a) whereas grafts in the lateral striatum have no effect on rotation (Dunnett et al., 1983; Dunnett et al., 1981b). Conversely, grafts reinnervating the lateral, but not dorsal, region of the striatum attenuate sensory deficits induced by 6-OHDA lesions (Björklund et al., 1980a; Dunnett et al., 1983; Dunnett et al., 1981a; Dunnett et al., 1981b).

Number of cells. It has been estimated that the yield of surviving dopaminergic neurons following grafting is only 0.1-1% of the total cell number initially implanted (Brundin et al., 1985b). Since the degree of rotational recovery is related to the number of surviving TH neurons, the number of cells implanted is of critical importance (Brundin et al., 1988a). For rat-to-rat allografts, 100-200 dopaminergic neurons are necessary for amphetamine-induced rotational recovery (Brundin et al., 1988a). Similarly, the degree of rotational recovery is also correlated with extent of reinnervation of the dorsal striatum (Björklund et al., 1980a; Dunnett et al., 1981a). Interestingly, at higher levels of neuronal

survival (Brundin et al., 1988a), striatal innervation (Björklund et al., 1980a), or DA release (Strecker et al., 1987), a plateau in behavioral recovery is reached with additional survival providing no further rotational benefit.

Specificity of tissue. Several lines of evidence demonstrate that the observed behavioral effects are dependent on the presence and survival of dopaminergic neurons within the striatum. Grafting of peripheral nerve (Freed et al., 1980), tectal tissue, cortical tissue (Freed et al., 1983), striatal tissue, or serotonergic cells derived from the raphe nucleus (Dunnett et al., 1988) does not produce behavioral recovery. Moreover, if the grafted tissue is removed (Björklund et al., 1980a), immunologically rejected (Brundin et al., 1989), or subsequently lesioned with 6-OHDA (Dunnett et al., 1988), behavioral recovery is lost, and rotation values return to pretransplant levels. The lesioning of the grafted neurons also results in a concomitant decrease in DA levels equal to that observed in lesion-only controls (Dunnett et al., 1988). Since lesioning of the graft causes loss of behavioral recovery, it may thus be concluded that it is the graft itself, rather than regenerative or trophic actions, which is responsible for the observed functional effects.

Functional aspects of grafted dopaminergic neurons.

Behavioral studies. As discussed above, intrastriatal nigral grafts have been shown to attenuate apomorphine and amphetamine induced rotational asymmetry, as well as sensory inattention (Björklund et al., 1980b; Björklund and Stenevi, 1979; Dunnett et al., 1983; Dunnett et al., 1981a; Dunnett et al., 1981b; Perlow et al., 1979). In addition, grafting of DA neurons decreases lesion-induced spontaneous rotation and restores normal behavior on a T-maze choice

task (Dunnett et al., 1983; Dunnett et al., 1981a). However, the extent of behavioral recovery afforded by neural grafting is not complete as all lesion-associated behavioral abnormalities are not restored following transplantation. The adipsia and aphagia induced by bilateral lesioning is not ameliorated (Björklund et al., 1980a; Dunnett et al., 1981b). In addition, paw reaching deficits caused by unilateral lesions are not improved by intrastriatal VM grafts (Abrous et al., 1993; Dunnett et al., 1987; Montoya et al., 1990) nor by grafts placed in the striatum and nucleus accumbens (Abrous et al., 1993). More recent studies using micrografting techniques have achieved greater striatal reinnervation (Nikkhah et al., 1994) as well as improvement on paw reaching tasks suggesting that the previously observed failure of behavioral compensation is likely due in part to insufficient striatal reinnervation produced by conventional macrografting techniques (Nikkhah et al., 1993a).

Neurochemical assessment of grafted neurons. Biochemical (Freed et al., 1980; Schmidt et al., 1983; Schmidt et al., 1982; Zetterström et al., 1986) and electrophysiological (Wuerthele et al., 1981) studies of VM cells implanted either into the ventricle, above the striatum, or as a cell suspension into the striatum indicate that grafted dopaminergic neurons are spontaneously active. Following ventricular grafting, DA has been found within grafts and at lower levels in the surrounding striatal tissue, suggesting that behavioral recovery is indeed mediated by DA release from the graft (Freed et al., 1980). However, with grafting to the lateral ventricle, it is possible that the DA effects in the striatum were due to diffusion rather than synaptic integration of graft fibers as fiber growth into the striatum was found to be limited (Freed et al., 1980). Post-mortem analyses have demonstrated that transplantation of fetal VM tissue

above or within the dorsal striatum restores striatal DA levels to approximately 13-18% of control levels (Schmidt et al., 1983; Schmidt et al., 1982). The DA turnover rate in the grafted striatum was found to be increased relative to that observed in the intact striatum, yet lower than that observed in lesion only controls (Schmidt et al., 1983; Schmidt et al., 1982). In parallel rotational studies, the concentration of DA was found to correlate with the degree of functional recovery, with a striatal DA level of only 3% of normal necessary to eliminate rotational asymmetry (Schmidt et al., 1983; Schmidt et al., 1982).

In vivo microdialysis measurements of DA and its metabolites within the grafted striatum further demonstrate transplant-derived restoration of DA synthesis and release (Strecker et al., 1987; Zetterström et al., 1986). Moreover, the grafted nigral neurons respond to dopaminergic drugs in the same manner as do intact nigral neurons. For example, amphetamine has been shown to increase DA release by grafted neurons (Zetterström et al., 1986). Microdialysis and electrophysiological analyses have demonstrated that the DA release by the grafts is regulated. As is characteristic of dopaminergic neurons, administration of the DA agonist apomorphine has been found to decrease DA release by the transplant, indicating an autoregulatory capacity of the grafted neurons, and addition of the DA reuptake inhibitor nomifensine increases extracellular DA, indicating the presence of functional reuptake sites on the grafted cells (Strecker et al., 1987). Similarly, administration of DA agonists has been found to decrease neuronal activity whereas DA antagonists increase activity (Wuerthele et al., 1981). Graft regulation is also suggested by the observation that behavioral recovery plateaus at high levels of cell survival, with further neuronal survival

providing no additional behavioral effect (Brundin et al., 1988a; Strecker et al., 1987).

Graft afferent and efferent connections. The observed functional recovery is associated with outgrowth of graft derived fibers into the striatum and the formation of synaptic connections between the graft and the host (Freund et al., 1985; Mahalik and Clayton, 1991; Mahalik et al., 1985). In addition to graft derived efferent fibers, there is an elaborate synaptic circuitry established within the graft itself (Bolam et al., 1987; Doucet et al., 1989), and anterograde tracer and electron microscopy studies have identified host afferents innervating intrastriatal VM grafts (Doucet et al., 1989; Mahalik et al., 1985). Afferents from the host cortex as well as a sparse distribution of serotonergic fibers from the host brainstem have been identified within grafts, and synaptic contacts with graft derived neurons have been observed (Doucet et al., 1989). Interactions with host striatal fibers appear to be limited to the graft-host interface, and the majority of the graft is not innervated by striatal afferents which normally project to the SN (Doucet et al., 1989; Freund et al., 1985). These findings suggest that the behavioral recovery and the regulation of grafted neurons may be mediated in part by host-graft interactions. Furthermore, the incomplete host-graft innervation may contribute to the lack of recovery on certain behavioral tasks following intrastriatal VM transplantation (Doucet et al., 1989).

Restoration of lesion induced changes at the cellular level. In addition to the ability to compensate for lesion induced behavioral abnormalities, grafted dopaminergic neurons are also able to reverse many lesion associated striatal receptor and gene expression changes, re-establishing the normal inhibitory or facilitory control by DA. The behavioral finding that fetal VM grafts reverse

receptor supersensitivity as measured by apomorphine induced rotation has been confirmed at the receptor level as the elevated binding of DA receptor ligands observed in lesioned rats is normalized by VM grafts (Dawson et al., 1991b; Freed et al., 1983; Rioux et al., 1991). Furthermore, fetal VM grafts have been shown to attenuate changes in striatal interneurons and striatal projection neurons which are associated with DA depletion. For example, the increased levels of preproenkephalin (Bal et al., 1993; Cenci et al., 1993) and proenkephalin mRNA (Mendez et al., 1993), as well as the decreased levels of preprotachykinin (substance P) and prodynorphin mRNA (Cenci et al., 1993; Mendez et al., 1993) associated with 6-OHDA lesioning are attenuated by intrastriatal VM grafts. Moreover, grafted DA neurons inhibit cholinergic neurons (Dawson et al., 1991a) as well as neuropeptide Y-expressing neurons (Moukhles et al., 1992) as is observed in the intact striatum. Grafted rats, like control rats, also exhibit increased striatal fos expression in response to amphetamine and decreased fos expression following apomorphine administration (Cenci et al., 1992). These regulatory effects are thought to be mediated by synaptic neurotransmission as well as by diffusion of DA from the grafted neurons as effects are observed beyond the area of reinnervation (Cenci et al., 1993; Cenci et al., 1992; Manier et al., 1991; Moukhles et al., 1992). While the mechanisms underlying graft-mediated functional recovery are incompletely understood, the re-establishment of synaptic circuitry and regulation at the cellular level are likely integral components for the restoration of function.

Xenotransplantation of fetal dopaminergic neurons. The studies reviewed above have provided significant information about the properties and

capabilities of transplanted dopaminergic neurons. Similar studies using human fetal tissue have been conducted in order to characterize the properties of transplanted human fetal DA neurons and to determine the optimal dissection and transplantation parameters for clinical use. In addition to defining the transplantation variables, xenografting into the rat striatum has been a useful means to address several issues of CNS immunology as described below.

The optimal donor age for human fetal tissue has been determined to be at approximately 6.5 - 9 weeks post-conception (Brundin et al., 1986). Human dopaminergic neurons grafted to the immunosuppressed or athymic adult rat have been shown to reinnervate the striatum, attenuate spontaneous and drug-induced rotational asymmetry (Brundin et al., 1986; Brundin et al., 1987; Strömberg et al., 1986), restore DA release (Brundin et al., 1987), and establish synaptic connections (Clarke et al., 1988; Strömberg et al., 1989). Graft-derived axons have been found to extend up to 10 mm, suggesting the long-distance growth potential of transplanted human fetal neurons in the adult parkinsonian brain (Wictorin et al., 1992). Moreover, these axons were found to exhibit target specificity, reinnervating only those areas normally innervated by dopaminergic neurons (Wictorin et al., 1992). The results from these human xenograft experiments, in combination with grafting fundamentals established from the allograft experiments, have provided the basis for the ongoing clinical trials of transplantation of human fetal tissue for the treatment of PD outlined above.

Immunological considerations for neurotransplantation.

Transplantation into the CNS raises questions regarding the potential for immunologic reactions against the grafted tissue. The genetic disparity between

the donor and the host as well as the immunologic status of the recipient at the time of grafting are critical determinants of graft fate. In addition, the grafting procedure and site of grafting may also influence survival (see e.g., Sloan et al., 1991; Widner, 1995 for review).

Basic immunology. The primary function of the host immune system is to distinguish between "self" and "non-self" in order to recognize foreign antigens and protect the host (see Lampson, 1987; Sloan et al., 1991; Widner, 1995; Widner and Brundin, 1988 for reviews). There are two main components of the immune system which are responsible for host defenses, classified either as cell-mediated (T cell) or antibody-mediated (B cell) responses. T cells normally recognize antigens in association with self-major histocompatibility complex (MHC) molecules, cell surface glycoproteins expressed on various cell types such as macrophages and microglia. Different subsets of T cells respond to antigens presented in association with different MHC molecules (MHC restriction). Specifically, cytotoxic T cells (CD8) recognize antigens in association with self-MHC I whereas helper T cells (CD4) recognize processed antigen presented by MHC II bearing cells. Following helper T cell binding to the antigen-MHC complex, the helper T cell is activated, resulting in the secretion of cytokines, such as interleukin-2 (IL-2), which serve to amplify the immune response by T cell proliferation and recruitment of additional cells as well as the promotion of antibody production. In allograft rejection, cell-mediated responses predominate whereas in xenografts, preformed antibodies may play a critical role in rejection (Sloan et al., 1991; Widner and Brundin, 1988). In addition to the aforementioned self-MHC mediated reactions, an additional mechanism may underlie graft rejection. If a cell expresses an MHC molecule which is similar, yet not identical

to self-MHC, then the host may recognize this as a self-MHC-antigen complex and an immune response may be initiated.

Immunologic considerations in CNS. The CNS is considered to be an immunologically privileged site as tissue grafted to the brain, if it is rejected, is rejected at a slower rate than similar tissue grafted to the periphery. While it was once believed that the CNS immunologic privilege was absolute, this privilege is only relative since under given circumstances, immune reactions do occur in the CNS.

Several factors unique to the CNS are thought to contribute to the relative immunologic privilege. The brain does not have a lymphatic drainage system, the route by which antigens normally travel to the lymph nodes. However, it has been shown that antigens in the brain do indeed access area lymph nodes (Widner et al., 1988). In addition, there are no designated antigen presenting cells in the brain which, as mentioned above, are necessary for T cell activation. However, several cell types within the CNS may act as antigen presenting cells under various circumstances (see Widner, 1993 for review). An additional mechanism contributing to the privileged status of the brain is the BBB which provides a barrier to the passage of molecules. However, activated lymphocytes are able enter the CNS (Wekerle et al., 1986). Yet another unique feature in the CNS is the paucity of MHC expression by neurons and glia (Lampson, 1987). However, under certain circumstances, including the trauma associated with grafting, MHC expression is induced (Lawrence et al., 1990; Mason et al., 1986; Neumann et al., 1995; Sloan et al., 1990).

Various transplantation combinations have been evaluated experimentally. Syngeneic grafts involve transplantation of tissue between

genetically identical subjects, whereas allogeneic grafts involve transplantation between individuals of the same species which differ genetically, and xenogeneic grafts are across species. As described below, the type of tissue grafted, as well as graft site, and implantation method (cell suspension versus solid tissue) impact subsequent immune responses and graft survival.

Allografting. Current clinical transplantation trials for PD involve allografting of human fetal tissue. As the donor and recipient will assuredly differ at major (MHC) and minor (non-MHC) transplantation loci, the grafted tissue will be immunologically incompatible with the host. However, the degree of immunologic reaction to CNS allografts is not clear, and thus the role of immunosuppressants in clinical transplantation is not precisely defined. Some trials use continuous immunosuppression, whereas others use short-term or no immunosuppression.

Experimental studies have addressed the outcome of grafts derived from an allogeneic source. In some studies, grafts which differ in either major or minor transplantation antigens have been found to survive with varying degrees of cellular infiltrates observed within the grafts (Freed et al., 1988; Low et al., 1983; Mason et al., 1986; Widner et al., 1989), whereas other studies have shown allogeneic graft rejection (Lawrence et al., 1990). Similarly, grafts differing in both major and minor transplantation antigens have been found to survive in some studies (Widner et al., 1989), yet more variable survival has been observed in other studies (Mason et al., 1986; Sloan et al., 1990). The variability in the outcome of these studies may be due in part to differences in transplantation sites as well as trauma associated with transplantation technique. It has been found that intraparenchymal grafts show improved survival relative to

intraventricular grafts, and grafting of solid tissue pieces causes more trauma at the implantation site than does intraparenchymal cell suspension grafting (Sloan et al., 1990).

The evaluation of cellular infiltrates associated with neuronal grafting has shown the presence of lymphocytic infiltrates and increased MHC expression (Duan et al., 1995a; Lawrence et al., 1990; Mason et al., 1986; Sloan et al., 1990). For example, in the case of intrastriatal cell suspension grafts, activated microglia, lymphocytes, and macrophages as well as increased expression of MHC I and MHC II antigens are observed at the graft site 4 days after transplantation (Duan et al., 1995a). This reaction has been found to subside by 6 weeks after grafting with no apparent deleterious effects on neuronal survival suggesting that the duration of MHC expression may be a predictor of subsequent immune response (Duan et al., 1995a). Notably, a similar mild cellular response is also found immediately following syngeneic grafting (Duan et al., 1995a; Mason et al., 1986).

An important caveat to consider in clinical transplantation is that patients may undergo staged bilateral transplantation procedures. Thus, the recipient may be immunized by the first graft, potentially jeopardizing survival of the second as well as the initial graft. This issue has been addressed experimentally by conducting sequential allografts in rats. Allografted rats receiving a subsequent syngeneic grafts have been reported to show no signs of graft rejection, however, allografted rats receiving a second allograft have been found to show either reduced survival of the second graft (Widner and Brundin, 1993), or similar survival yet increased MHC II staining at the site of the second graft (Duan et al., 1993). While no deleterious effects were observed on the first graft,

rats receiving allografts have been found to be immunized by the grafted tissue, as shown by increased antibody titers and by increased numbers of alloreactive cells (Widner and Brundin, 1993). The status of the host at the time of transplantation has been demonstrated to affect graft survival as transplants to rats systemically sensitized via a skin graft prior to grafting fail to survive (Duan et al., 1995b; Mason et al., 1986). While the peripheral detection of antibodies has not been found to correlate with the likelihood of graft rejection, it does however indicate that the host has been immunized.

Xenotransplantation. The use of xenogeneic tissue for transplantation would circumvent many of the practical and ethical issues associated with the transplantation of human fetal tissue. While the need for immune suppression in allografting is not yet clear, it is a requirement in the setting of xenografting as transplantation between species rarely results in sustained graft survival in the absence of immunosuppression (Pakzaban and Isacson, 1994). Although xenograft survival has been observed in non-immunosuppressed hosts in a few instances (Björklund et al., 1982; Brundin et al., 1985c; Daniloff et al., 1985), Cyclosporine A (CyA) has clearly been demonstrated to enhance xenograft survival in numerous transplantation paradigms (Brundin et al., 1985c; Brundin et al., 1988b; Finsen et al., 1988b; Inoue et al., 1985). Indeed, meta-analysis of xenotransplantation studies has shown that CyA treatment is the most important factor to achieve xenograft survival, improving graft survival from 30% in non-immunosuppressed hosts to 74% in the presence of continuous CyA treatment (Pakzaban and Isacson, 1994). CyA inhibits T cell activation, and the effectiveness of CyA indicates the involvement of T cell-mediated immune responses in xenograft rejection. Furthermore, evidence of cell mediated

rejection is found in analyses of cell infiltrates following xenografting in non-immunosuppressed hosts (Duan et al., 1995a; Finsen et al., 1988a; Finsen et al., 1991; Mason et al., 1986).

In contrast to the relatively mild immune response associated with cell suspension grafts of syngeneic or allogeneic tissue, the response to xenogeneic tissue grafted in the non-immunosuppressed host is sustained and more vigorous (Duan et al., 1995a; Finsen et al., 1991). Rejection of solid grafts in the CNS has been considered analogous to allograft rejection in the periphery due to the similar timecourse and characterization of the cellular infiltrate (Finsen et al., 1988a). An inflammatory infiltrate in solid grafts has been noted as early as three days after grafting, peaking at 3 weeks after transplantation (Finsen et al., 1991). Early after grafting, MHC I expression has been found to be increased, whereas 2 weeks after grafting MHC II was more highly expressed (Duan et al., 1995a). While the characterization of the cellular infiltrate present during xenograft rejection has revealed predominantly T cells (Finsen et al., 1990), it has also been suggested that preformed antibodies and complement activation may be involved (Duan et al., 1995a; Mason et al., 1986). Indeed, marked tissue damage can be noted as early as 4 days after xenografting in some studies (Duan et al., 1995a).

Immunosuppressive agents. As discussed above, the immunosuppressive drug regimen used in clinical trials of fetal neuronal transplantation is variable, and the optimal approach is not yet known. In general, combinations of three immunosuppressive agents are routinely used, either CyA alone or CyA in combination with corticosteroids and/or azathioprine. CyA prevents T cell activation by inhibiting the synthesis of IL-2 and its receptor. Corticosteroids

inhibit cytokine production and T cell proliferation, and the antimitotic agent azathioprine inhibits clonal expansion (Suthanthiran and Strom, 1994).

Conventionally, CyA has been employed in experimental studies of xenotransplantation. However, as indicated above, this agent affords less than optimal graft survival as on average only 74% of CyA treated grafts survive. Only recently have other agents been evaluated experimentally in the context of neuronal xenografting. The corticosteroid methylprednisolone has been shown to be as effective as CyA in preventing rejection of mouse to rat cell suspension xenografts (Duan et al., 1996). In addition, triple drug therapy using CyA, prednisolone and azathioprine is also effective in preventing graft rejection (Pedersen et al., 1995). Further assessment of immunosuppressive agents in experimental conditions is clearly necessary to determine the optimal drug combinations for CNS transplantation. Moreover, evaluation of the rejection process may facilitate the development of novel approaches to immunosuppression.

Aims of present studies

The following studies were aimed at determining methods of neuroprotection and neuronal replacement in rat models of PD. Specifically, the protective effects of the neurotrophin BDNF were evaluated histologically and neurochemically in an MPP+ model of SN degeneration. In efforts to address the mechanism of NTF mediated protection, the ability of NGF to prevent the generation of the oxidant peroxynitrite was evaluated in an animal model of mitochondrial toxicity. As an alternative to neuroprotective strategies, neuronal replacement was evaluated by transplanting fetal porcine VM into the DA depleted striatum of 6-OHDA lesioned rats and assessing graft-mediated functional recovery and striatal reinnervation.

CHAPTER II

IMPLANTED FIBROBLASTS GENETICALLY ENGINEERED TO PRODUCE BRAIN-DERIVED NEUROTROPHIC FACTOR PREVENT 1-METHYL-4-PHENYLPYRIDINIUM TOXICITY TO DOPAMINERGIC NEURONS IN THE RAT

Abstract

The trophism of BDNF for dopaminergic cells in culture has led to significant interest in the role of BDNF in the etiology and potential treatment of PD. In this study, we produced nigral degeneration by infusing MPP+, a mitochondrial complex I inhibitor and the active metabolite of MPTP, into the rat striatum. The subsequent loss of nigral neurons was presumably due to mitochondrial toxicity after MPP+ uptake and retrograde transport to the SN. Immortalized rat fibroblasts were engineered to secrete human BDNF and implanted near the SN 7 days prior to striatal MPP+ infusion. BDNF-secreting fibroblasts were found to markedly increase nigral dopaminergic neuronal survival when compared to control fibroblast implants. The observation that BDNF prevents MPP+ induced dopaminergic neuronal degeneration in the adult brain has significance for the treatment of neurodegenerative disorders which may involve mitochondrial dysfunction, such as PD.

Introduction

The range of neuroprotective effects for any putative neurotrophic molecule must be determined in *in vivo* models of neurodegeneration (Anderson et al., 1988; Frim et al., 1993b; Knusel et al., 1992; Kromer, 1987; Schumacher et al., 1991; Sendtner et al., 1992a; Yan et al., 1992) before practical application of NTF neuroprotection can be contemplated (Morgan, 1989). Of particular interest are NTFs found to protect *in vivo* against neuronal death caused by mechanisms similar to those thought to occur in human neurodegenerative disorders, such as NGF protection against HD-like lesions of the striatum (Frim et al., 1993b; Schumacher et al., 1991) or CNTF protection in a mouse model of motor neuron degeneration (Sendtner et al., 1992b). BDNF (Barde et al., 1982; Hofer and Barde, 1988) promotes the survival of fetal mesencephalic dopaminergic cells in culture (Hyman et al., 1991). *In vivo*, BDNF protects cholinergic neurons from degeneration after fimbria-fornix lesions (Knusel et al., 1992) and motoneurons from degeneration after axotomy (Sendtner et al., 1992a; Yan et al., 1992).

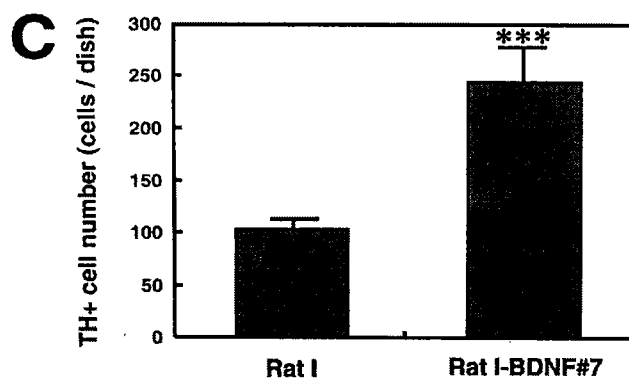
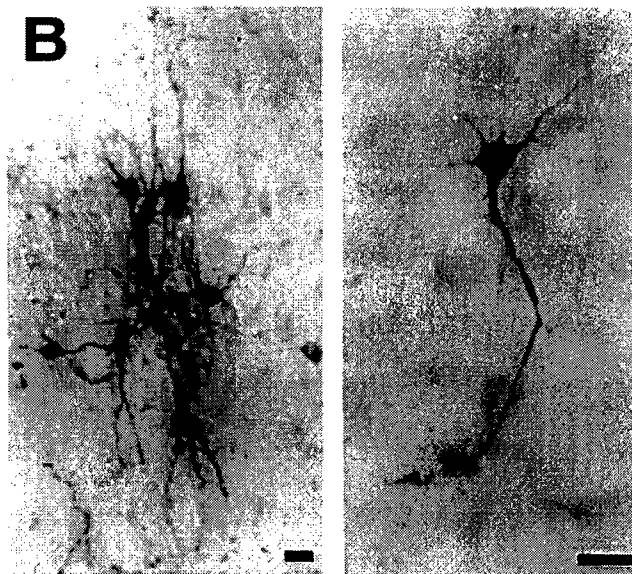
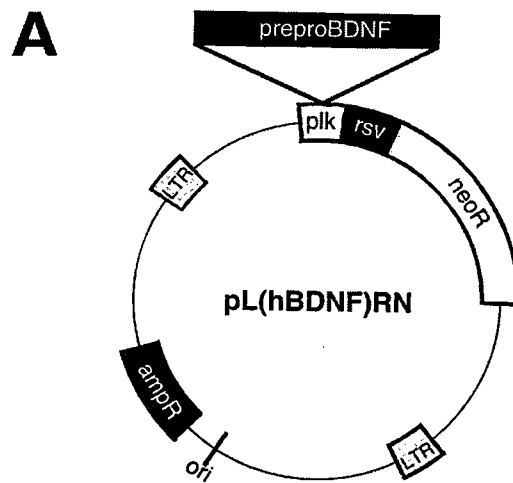
The cellular insults that cause some human neurodegenerative diseases may be only partially modeled by axotomy-induced cell death (Lams et al., 1988; Sofroniew et al., 1990; Sofroniew and Isacson, 1988). Other insults to cellular integrity, such as impaired energy metabolism, may more accurately model the degeneration seen in HD or PD (Beal, 1992; Choi, 1988; Simpson and Isacson, 1993; Wallace, 1992). Indeed, evidence for abnormalities in mitochondrial function have been found in patients with both of these disorders (Brennan et al., 1985; Parker et al., 1989). A well described animal model of PD is produced by the systemic injection of MPTP into mice or primates (Burns et al., 1983; Heikkilä

et al., 1984a). MPTP, which is metabolized to the mitochondrial complex I inhibitor MPP⁺, causes dopaminergic cell death in the SNc, presumably due to mitochondrial impairment (Nicklas et al., 1985; Nicklas et al., 1987). BDNF has been shown to protect dopaminergic neurons against MPP⁺ toxicity in culture (Hyman et al., 1991). In the present study, we used a model of SNc dopaminergic neuronal degeneration based on MPP⁺ mediated mitochondrial impairment to test whether BDNF can protect dopaminergic neurons against such insults *in vivo*.

Materials and methods

Genetic engineering and selection of a BDNF-secreting fibroblast cell-line. Cells used for implantation were generated by infection of an immortalized rat fibroblast cell line (Rat I, initially provided by M. Rosenberg, UCSD) with a retrovirus vector carrying a human cDNA encoding a full-length preproBDNF precursor identified by sequence analysis (provided by G. Walz and B. Seed, Massachusetts General Hospital) as described (Frim et al., 1993a). Briefly, the preproBDNF cDNA was cloned into the polylinker region of a retroviral plasmid (pL(X)RNL, provided by M. Rosenberg, UCSD) under control of the Moloney murine leukemia virus long terminal repeat promoter in a construct containing the Tn5 neomycin resistance gene, as shown in Figure 4A. Recombinant plasmid was transfected into ψ -2 ecotropic retrovirus producer cells by calcium shock, and resultant ψ -2-BDNF cells were selected under G418. Retrovirus particles were isolated from the highest producer, and Rat I cells were infected by repeated incubations with ψ -2-BDNF conditioned media in the presence of

Figure 4. Engineering of BDNF-secreting fibroblasts for transplantation. A. Schematic of the pL(BDNF)RNL retroviral plasmid used for transfection of the ψ -2 producer cells. PreproBDNF indicates the DNA fragment containing a full-length human BDNF cDNA inserted into the polylinker region of the plasmid; rsv, Rous sarcoma virus internal promoter; neoR, neomycin resistance gene; LTR, Moloney murine leukemia (5') or sarcoma (3') virus long terminal repeat; ampR, ampicillin resistance gene; ori, E. coli origin of replication. Plasmid is not drawn to scale. B. Photomicrographs of co-culture of TH immunopositive fetal VM neurons with confluent plating of fibroblasts. (i) Low power view of cluster of TH immunopositive cells plated on confluent Rat I-BDNF #7 cells. Note neuronal morphology and neuritic extensions; (ii) higher power view of individual TH immunopositive neuron plated on Rat I-BDNF #7 cells. Scale bars = 20 μ in both panels. C. Bar graph of effects of co-culture with Rat I fibroblasts or Rat I-BDNF #7 fibroblasts on TH immunopositive fetal mesencephalic neurons after 5 days *in vitro*. Error bars represent S.D. ***, significant to $p < 0.001$ by t-test.



polybrene. Rat I-BDNF sub-clones were selected under G418 and then screened for their level of BDNF mRNA production by Northern analysis and for their level of BDNF secretion by an *in vitro* rat dorsal root ganglion (DRG) neurite outgrowth bioassay, as previously described (Frim et al., 1993d).

Co-culture of BDNF-secreting fibroblasts with fetal ventral mesencephalic neurons. In order to fully confirm the secretion of BDNF by the selected cell-line (Rat I-BDNF #7), VM neurons were dissected and plated on confluent cultures of Rat I-BDNF #7 cells in an identical manner to that previously described (Hyman et al., 1991). After 5 days, cultures were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) for 15 min and then immunostained for TH (Figure 4B) as described below for tissue sections. The number of cells per plate was counted in a uniform square centered in the plate (to avoid edge artifact) of approximately 256 mm². Plates containing areas of non-confluence were excluded from the cell counting analysis. TH positive cell counts were averaged from three Rat I confluent plates as control and seven Rat I-BDNF #7 confluent plates and expressed as mean \pm S.D. These two conditions were compared by t-test to confirm the bioactivity of the secreted BDNF (Figure 4C).

Cell preparation and implantation in substantia nigra degeneration model. Cells were harvested by trypsinization and injected as a suspension in PBS, with 1.0 mg/l CaCl₂, 1.0 mg/l MgCl₂, and 0.1% glucose. Cell count and viability were assessed by trypan blue dye exclusion before intracerebral

injection into rat hosts. Rat I cells not altered by retroviral infection were injected into control animals.

Under pentobarbital anesthesia (65 mg/kg), 8 male Sprague-Dawley rats (300-350 gm) were grafted with Rat I fibroblast cells (BDNF[-]) and 8 rats were grafted with Rat I-BDNF #7 cells (BDNF[+]) (Figure 5). Using a Kopf rat stereotactic frame, burr holes for grafting into the dorsal tegmentum of the mesencephalon were made at coordinates calculated from bregma as AP -5.3, L -2.4. Injections were made using a 10 μ l Hamilton syringe at V -6.0 calculated from the dura. A total of 2×10^5 cells were infused over 2 min in a volume of 2 μ l; the needle was withdrawn after a 1 min pause.

Seven days after cell implantation, animals received MPP+ infusions into the striatum (Storey et al., 1992). In a manner identical to that described above, burr holes for injection were calculated from bregma and made at AP +1.0, L -2.5; over 1 min, 100 nmol of MPP+ in 1 μ l was infused at V -4.5 calculated from the dura. A single animal in the BDNF[-] group did not survive the second surgical procedure.

Animals were sacrificed 7 days after lesioning while under deep barbiturate anesthesia by cardiac puncture and perfusion with cold heparinized saline, followed by 4% paraformaldehyde in PBS. Brains were removed, postfixed in 4% paraformaldehyde in PBS at 4 $^{\circ}$, then cryoprotected in 30% sucrose in PBS until equilibration. Serial sections cut at 40 μ m on a freezing microtome were stained with cresyl violet (Nissl) or immunostained for TH (East Acres Biologicals, Southbridge, MA) at a primary antibody dilution of 1:3000.

Figure 5. Schematic diagram of experimental paradigm. Rats were grafted with BDNF[+] or BDNF[-] cells dorsal to the SN 7 days prior to striatal infusion of MPP+. Animals were evaluated 7 days after MPP+ administration.

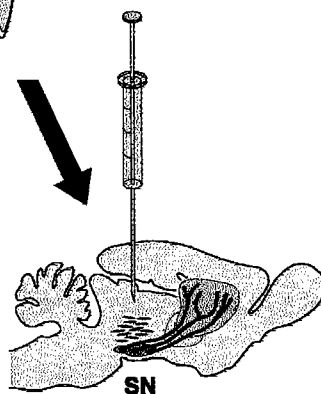
NTF[+] or
NTF[-]
fibroblasts



Cell suspension



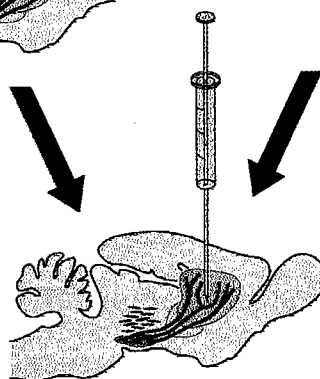
Implantation of
cell suspension



MPP+



Striatal infusion
of MPP+



Morphometric procedures. The maximal cross-sectional area of the striatal lesion, as seen by characteristic neuronal loss after cresyl violet staining, was determined for each animal and measured as described (Schumacher et al., 1991). SNc neurons were visually counted bilaterally both on Nissl-stained sections and on TH-immunostained sections. Since all SNc cell counts are expressed as a percentage of surviving cells relative to the control cell number on the non-MPP+-injected side, no correction factors were used. The TH immunopositive SNc cell counts were independently confirmed by two observers, one of whom was blinded to the treatment regime.

Statistical analysis. A ratio of lesioned side to non-lesioned side (grafted side to non-grafted side) Nissl stained and TH immunostained SNc neurons was determined for each animal. These ratios (expressed as percent surviving SNc neurons on the lesioned side) were compared by t-test for BDNF[-] and BDNF[+] animals using the program Statview 4.0 (Abacus). In order to assess the local and retrograde effects of infused MPP+, regression analysis of measured striatal lesion size versus percent surviving SNc neurons was also performed using the Statview 4.0 program for Nissl stained and TH immunostained cell counts in both BDNF[-] and BDNF[+] implanted animals.

Results

BDNF-secreting fibroblasts. Immortalized fibroblasts were engineered to secrete BDNF using techniques previously employed for constructing NGF-secreting fibroblasts (Frim et al., 1993a). As described (Frim et al., 1993d),

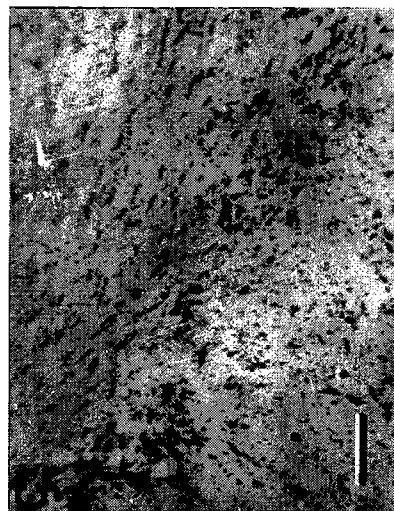
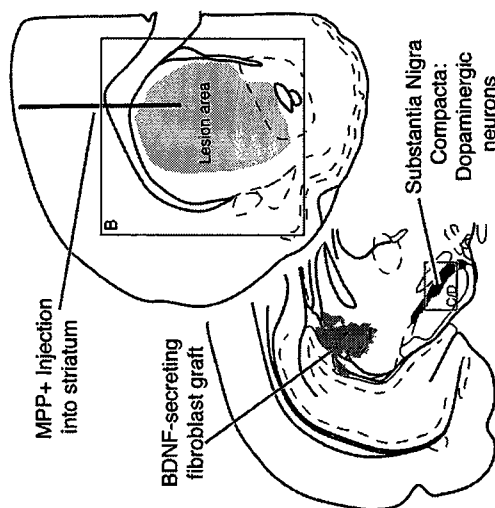
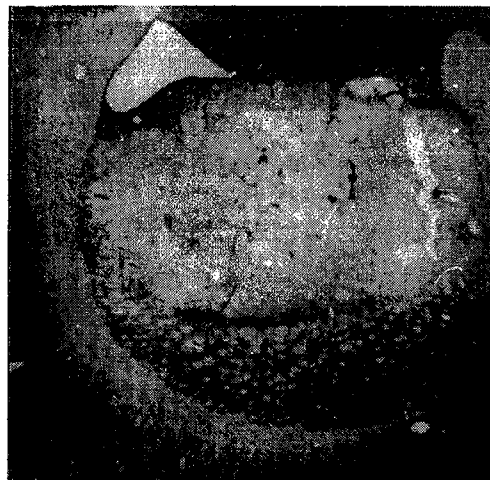
transgenic BDNF mRNA synthesis was screened by Northern blotting of total RNA from Rat I-BDNF cells followed by hybridization to a radiolabeled human preproBDNF antisense cRNA probe. This analysis revealed an mRNA species of similar size to previously described preproNGF-neoR mRNA transcribed in the identical vector backbone (Wolf et al., 1988).

BDNF secretion by the engineered cells was initially screened by testing Rat I-BDNF conditioned media in a neurite outgrowth assay using neonatal rat DRG explants. Conditioned media from Rat I-BDNF #7 cells, at a dilution of 1:130, supported DRG survival and neurite outgrowth for 7 days after explantation. Conditioned media from uninfected Rat I cells at a dilution of 1:10 did not support DRG outgrowth or survival after 7 days.

The presence of BDNF secretion by the highest producing selected sub-clone was fully confirmed by co-culture of Rat I-BDNF #7 cells with TH immunopositive fetal VM neurons (Figure 4B) as previously described (Hyman et al., 1991). Co-culture of fetal mesencephalic neurons with Rat I cells supported 104 ± 9 TH immunopositive cells per plate as compared to co-culture with Rat I-BDNF #7 cells, which supported 241 ± 35 TH immunopositive cells per plate. This approximately 140% increase in TH immunopositive cells per plate (significant to $p < 0.001$ by t-test) is highly consistent with previously observed specific biological effects of BDNF on fetal mesencephalic neurons supported in culture from 2 to 8 days (Hyman et al., 1991).

Striatal infusion of MPP+ and SNc degeneration. The placement of BDNF-secreting grafts near the SNc and the overall strategy of inducing SNc degeneration by striatal MPP+ infusion is schematically represented in Figure 6. The presence of significant striatal lesions due to infusion of 100 nmol of MPP+

Figure 6. Model of SNc degeneration after striatal MPP+ infusion. Panel A depicts a schematic overview of the SNc degeneration model. The anterior section (approximately bregma +1.0) shows a needle tract for MPP+ infusion into the striatum and a tracing of the lesion 7 days later. The boxed area corresponds to that shown in Panel B, a cresyl violet stained section through the striatum showing a typical lesion induced by MPP+ infusion. Note the cell loss and shrunken fiber bundles in the area of the lesion. The lower section of Panel A (approximately bregma -5.0) shows an example of fibroblast graft placement and indicates the location of the SNc in relation to the graft. The boxed area on this section represents the approximate areas shown in Panels C and D. Panels C and D depict cresyl violet (Nissl) stained sections taken from a control animal implanted with a non-secreting (BDNF[-]) graft. Panel C represents the SNc ipsilateral to the striatal MPP+ infusion where neuronal cell number is markedly reduced when compared to Panel D, the non-lesioned contralateral SNc from the same cresyl violet stained section. Size bar, 100 μ in panels C and D.



was confirmed in every animal by characteristic neuronal loss after cresyl violet staining (Figure 6B). The lesion size was quantifiable by selection and measurement of a maximal cross-sectional lesion area for each animal, as has been performed previously for excitotoxic lesions in the striatum (Frim et al., 1993b; Schumacher et al., 1991). This analysis revealed that there was no difference between the size of MPP+ induced striatal lesions in the BDNF[-] ($5.46 \pm 2.61 \text{ mm}^2$; mean \pm S.D.) and BDNF[+] groups ($5.57 \pm 2.02 \text{ mm}^2$) indicating that the infused MPP+ had the same potential toxicity in both treatment groups.

Effects of BDNF-secreting grafts on SNc cell survival. Graft placement was confirmed on Nissl stained sections, though both the BDNF[-] and BDNF[+] grafts tended to extend out from the injection tract in the mesencephalic tegmentum and into the dorsal and ventral paramesencephalic cistern on the side of the implantation (Figure 6A). For this reason it was impossible to obtain an accurate estimate of graft size in every animal; however, no obvious differences were noted between the overall amount of surviving BDNF[-] and BDNF[+] fibroblasts at the time of sacrifice. In particular, all animals seemed to have a significant number of implanted fibroblasts either within the mesencephalon or adjacent to the midbrain in the paramesencephalic cistern. This placed grafted cells within 1 to 2 mm of the SNc on the grafted side in all animals.

Cell counting of Nissl stained neurons within the lesioned (Figure 6C) and non-lesioned (Figure 6D) SNc of animals grafted with BDNF[-] cells showed a 53% decrease in neurons on the lesioned side, to $47 \pm 11\%$ of the non-lesioned side, consistent with previous observations of SNc degeneration in this model (Srivastava et al., 1993). There was a significantly greater reduction in TH

immunopositive cells than in Nissl stained neurons ($p < 0.017$) in the SNc on the side of the striatal lesion in BDNF[-] implanted animals, to $35 \pm 15\%$ (Figure 7). Particularly noteworthy is a very high inverse correlation ($r = 0.921$, $F = 27.957$, $p < 0.003$) between striatal lesion cross-sectional areas and percent Nissl stained SNc neurons on the side of the lesion in animals implanted with BDNF[-] grafts. This emphasizes the utility of this model for studying MPP+ mediated SNc degeneration. The correlation between striatal lesion sizes and TH immunopositive SNc cells approached significance ($r = 0.709$, $F = 5.053$, $p < 0.075$) (Figure 8).

Placement of BDNF[+] grafts near the SNc on the lesioned side resulted in a significant ($p < 0.001$) increase in the percentage of surviving Nissl stained neurons to $86 \pm 18\%$, and rendered the correlation between striatal lesion size and SNc cell survival not significant ($r = 0.394$, $F = 0.919$, $p = 0.382$). The effect of the BDNF-secreting grafts on TH cell survival was also dramatic ($p < 0.001$) in that BDNF[+] grafts promoted the survival of $83 \pm 22\%$ of the TH immunopositive SNc cells on the side of the lesion (Figure 7B). The magnitude of these changes can be seen graphically in Figure 7D. As observed for the counts of Nissl stained neurons, BDNF[+] grafts reduced the inverse correlation between striatal lesion size and TH immunostained surviving cells to $r = 0.321$ from $r = 0.709$ in the BDNF[-] implanted animals. The regression analyses of striatal lesion sizes on percentage SNc Nissl stained neuronal survival and SNc TH immunostained cell survival are depicted graphically in Figure 8.

Figure 7. Effects of BDNF-secreting fibroblast implants on dopaminergic cell degeneration in the SNc. Depicted are TH immunostained cells found in the SNc (i) ipsilateral to striatal MPP+ infusion with a BDNF[-] graft (Panel A), (ii) contralateral to striatal MPP+ infusion in with a BDNF[+] graft (Panel B), and (iii) ipsilateral to MPP+ infusion in a BDNF[+] implanted animal (Panel C). There is a marked reduction in TH immunopositive cells after MPP+ infusion in the BDNF[-] implanted animals (Panel A) when compared to a section at a similar level in the SNc contralateral to an MPP+ infusion (Panel B). Relative to the BDNF[-] animal (Panel A), the number of SNc TH immunopositive cells spared ipsilateral to the striatal MPP+ infusion in the BDNF[+] implanted animal (Panel C) is markedly increased and seems to approach the corresponding non-lesioned SNc in Panel B. Panel D displays a graphical representation of the effects of the BDNF[-] and BDNF[+] grafts on cell sparing in the SNc, both for cresyl violet (Nissl) stained neurons as well as TH immunostained neurons (mean \pm SD). Cell survival is expressed as a percentage of the number of SNc neurons found contralateral to the MPP+ infusion. Scale bar in Panel B, 100 μ ; Panels A, B, and C are of identical magnification; *, difference significant to $p < 0.001$.

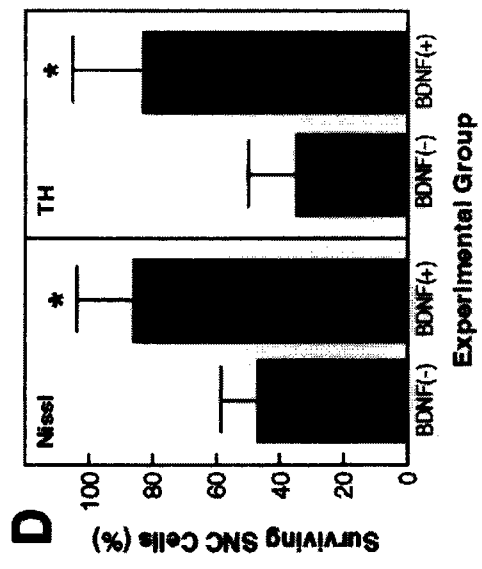
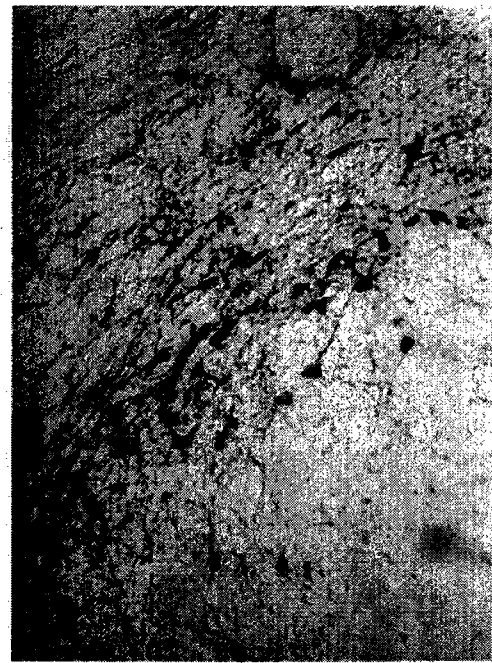
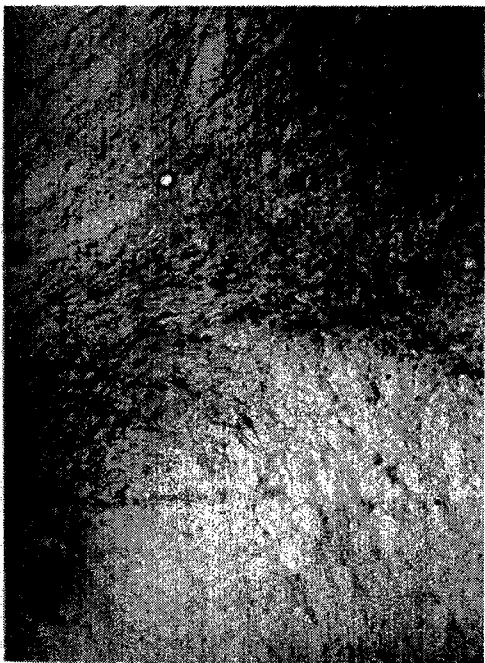
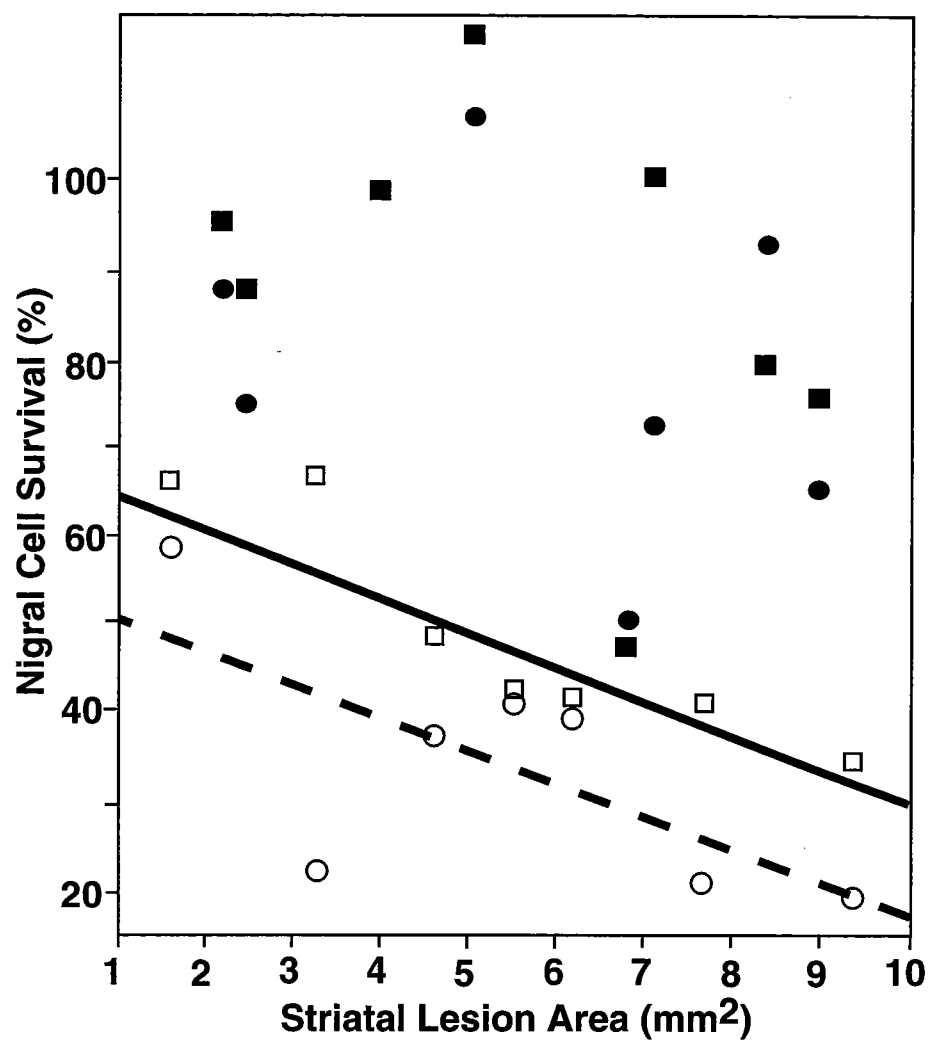


Figure 8. Regression analysis graph of striatal lesion maximal cross-sectional area versus cell survival in BDNF[-] and BDNF[+] implanted animals. This figure shows a summary of data points for each animal analyzed in this study. The squares represent SNc Nissl stained neuronal counts, the circles represent SNc TH immunopositive cell counts. Cell survival in the SNc ipsilateral to the MPP+ infusion within each animal, expressed as a percentage of the unaffected contralateral side, is graphed versus maximal striatal cross-sectional lesion area for BDNF[-] (open circles and squares) and BDNF[+] (closed circles and squares) animals. The unbroken line represents the regression line ($r = 0.921$, $F = 27.957$) of cresyl violet stained neurons in the SNc ipsilateral to the MPP+ infusion against striatal lesion size in control (BDNF[-]) animals. The broken line represents the corresponding regression for TH immunopositive SNc cells ($r = 0.709$, $F = 5.053$). Implantation of BDNF[+] grafts, aside from markedly increasing the percentage cell survival (all closed circles and squares lie above the BDNF[-] regression lines), resulted in no correlation between cell survival and the striatal lesion sizes ($r = 0.394$ for cresyl violet stained SNc neurons; $r = 0.321$ for TH immunostained neurons). This implies that the BDNF[+] cell counts, as shown by the closed circles and squares, now depend on variables independent of the striatal lesion sizes, such as degree of BDNF delivery.



Discussion

We have constructed immortalized rat fibroblasts capable of synthesizing and secreting transgenic human BDNF in amounts adequate to support explanted DRG neurite extension and dopaminergic neuronal survival in culture. Implantation of these fibroblasts near the SN in rats 7 days before striatal infusion of MPP+ significantly protected against subsequent SNc TH immunopositive neuronal death. The increased number of TH immunopositive cells in BDNF[+] animals reflects actual cell sparing, as opposed to changes in TH immunoreactivity, since the number of Nissl stained SNc neurons was also markedly reduced in BDNF[-] animals, consistent with neuronal death. Nissl stained neurons were significantly spared in BDNF[+] animals when compared to BDNF[-] controls.

In addition to a local lesion, infusion of MPP+ into rat striatum causes a profound loss of dopaminergic SNc neurons (Srivastava et al., 1993; Storey et al., 1992). This is presumably due to the uptake of MPP+ by dopaminergic terminals in the striatum and retrograde transport to the SNc (Campbell et al., 1990). In the present study, the loss of SN neurons was highly correlated with striatal lesion size. This correlation between striatal lesion size and SNc cell loss implies a similar cause for both striatal and SNc neuronal death. Since other insults that cause massive striatal excitotoxic lesions, such as toxic levels of NMDA-receptor agonists, do not cause appreciable SNc cell loss (Isacson et al., 1985), the cause of the SNc degeneration would likely be direct MPP+ effects on SNc neurons, not an indirect effect from the striatal neuronal death. The neuronal death in the SN is likely caused by mitochondrial blockade after retrograde transport of MPP+

from striatum to SNc and therefore acutely models the dopaminergic cell loss seen in PD. Interestingly, patients with PD can manifest mitochondrial defects (Parker et al., 1989; Shoffner et al., 1991), or more specifically, a deficiency in mitochondrial complex I activity (Schapira et al., 1990).

The protective effects of BDNF on survival of dopaminergic neurons in this model likely reflect a trophic influence on neuronal function, perhaps through increased mitochondrial efficiency or by induction of cellular protective mechanisms. Mechanisms of neurotrophin-mediated neuroprotection in the adult brain are poorly understood. NGF, the most extensively investigated neurotrophin, is known to modulate a number of intracellular events, including changes in intracellular calcium stores (Johnson et al., 1992), Na^+/K^+ ATPase activity (Sendtner et al., 1988), Cl^- flux (Rothman, 1985) and induction of the peroxidative enzyme, catalase, both in culture (Jackson et al., 1990) and *in vivo* (Frim et al., 1994b). BDNF protects cultured neuroblastoma cells against MPP⁺ and 6-hydroxydopamine toxicity, and induces a 100% increase in the activity of glutathione reductase, a protective enzyme (Spina et al., 1992). However, BDNF had no effect on catalase activity in that system.

NGF and BDNF share significant homology (Leibrock et al., 1989), both are able to bind to a low affinity neurotrophin receptor (Rodriguez-Tebar et al., 1990), and both bind with high-affinity to specific members of the *trk*-family of receptors (Kaplan et al., 1991a; Soppet et al., 1991; Squinto et al., 1991). It seems possible that BDNF may also mediate similar intracellular changes to those described for NGF. There also exists a level of specificity in neuronal populations for neurotrophin activity, for example, NGF does not protect cultured dopaminergic neurons against MPP⁺ toxicity (Hyman et al., 1991).

bFGF, on the other hand, does have some protective effects against MPTP toxicity in the mouse (Otto and Unsicker, 1990) but does not share homology with BDNF. The suggestion has been made that bFGF may cause glial cells to secrete neurotrophic factors which in turn effect neuronal protection (Engle and Bohn, 1991). These observations imply either multiple, complementary protective pathways for neurotrophic support, or a final common pathway, which can be activated through a number of receptors.

In vivo, BDNF protects against cholinergic neuronal degeneration after fimbria-fornix lesioning (Knusel et al., 1992), and ameliorates motor neuron death after axotomy (Sendtner et al., 1992a; Yan et al., 1992). However, previous investigations of *in vivo* effects of BDNF failed to show any protective effects on SNc neurons after medial forebrain bundle transection (Knusel et al., 1992). Our paradigm investigated BDNF amelioration of mitochondrial impairment in neurons, perhaps a more limited neurodegenerative insult than proximal axotomy. The cellular response to axotomy is complex (Lams et al., 1988; Sofroniew et al., 1990; Sofroniew and Isacson, 1988) and may not model the neuronal loss seen in neurodegenerative conditions.

Our observations that BDNF can protect dopaminergic neurons in the SNc against mitochondrial blockade indicate that adult SNc neurons still respond to neurotrophic input and perhaps require neurotrophic support for survival, particularly during aging, when mitochondrial function deteriorates (Wallace, 1992). The finding of protective effects of CNTF on dopaminergic neurons *in vivo* also supports the idea of neurotrophic input as means to prevent lesion-induced degenerative changes in the dopaminergic system (Hagg and Varon, 1993). Insufficient neurotrophic input in the adult, or failure to increase neurotrophic

support as mitochondrial function fails, may render SNc neurons more vulnerable to environmental toxins or to potentially toxic conditions within the SNc (Isacson, 1993). In such a case, replacement or supplementation of endogenous trophic support with BDNF input may increase the likelihood for SNc neuronal survival in a state of mitochondrial deficiency or oxidative stress.

ACKNOWLEDGEMENT

All studies presented in this chapter were conducted by the candidate with the exception of the cell-line construction and characterization which were done by David Frim in collaboration with Dr. Xandra Breakefield's laboratory, Neurogenetics Unit, Massachusetts General Hospital. This material has been published as:

Frim, D. M., Uhler, T. A., Galpern, W. R., Beal, M. F., Breakefield, X. O., and Isacson, O. (1994). Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. *Proc. Natl. Acad. Sci. USA* 91, 5104-5108.

CHAPTER III

CELL-MEDIATED DELIVERY OF BRAIN-DERIVED NEUROTROPHIC FACTOR ENHANCES DOPAMINE LEVELS IN AN MPP+ RAT MODEL OF SUBSTANTIA NIGRA DEGENERATION

Abstract

BDNF promotes the survival of fetal mesencephalic dopaminergic cells and protects dopaminergic neurons against the toxicity of MPP+ *in vitro*. Supra-nigral implantation of fibroblasts genetically engineered to secrete BDNF attenuates the loss of SNc dopaminergic neurons associated with striatal infusion of MPP+ in the adult rat. Using this MPP+ rat model of nigral degeneration, we evaluated the neurochemical effects of supranigral, cell-mediated delivery of BDNF on SN DA content and turnover. Genetically engineered BDNF-secreting fibroblasts (~12 ng BDNF/24h) were implanted dorsal to the SN 7 days prior to striatal MPP+ administration. The present results demonstrate that BDNF-secreting fibroblasts, as compared to control fibroblasts, enhance SN DA levels ipsilateral as well as contralateral to the graft without altering DA turnover. This augmentation of DA levels suggests that local neurotrophic factor delivery by genetically engineered cells may provide a therapeutic strategy for preventing neuronal death or enhancing neuronal function in neurodegenerative diseases characterized by dopaminergic neuronal dysfunction such as PD.

Introduction

The trophic support and neuroprotection afforded by neurotrophic factors in *in vitro* systems and in *in vivo* neurodegeneration models indicate the possible therapeutic applicability of these factors to neurodegenerative disorders (Hefti, 1994; Jelsma and Aguayo, 1994; Lindsay et al., 1993). NGF, the most extensively characterized neurotrophin, protects against cholinergic neuronal degeneration induced by fimbria-fornix transection (Hefti, 1986; Kromer, 1987; Rosenberg et al., 1988; Williams et al., 1986). More recently, GDNF as well as BDNF have shown neuroprotective effects in animal models of PD (Beck et al., 1995; Frim et al., 1994a; Tomac et al., 1995a)

The effects of the neurotrophin BDNF on fetal VM dopaminergic neurons was first demonstrated *in vitro* (Hyman et al., 1991; Hyman et al., 1994; Knusel et al., 1991). In addition to promoting survival of dopaminergic neurons in culture, the administration of BDNF to the intact adult rat brain is associated with significant behavioral and neurochemical alterations. BDNF (12 $\mu\text{g}/\text{d}$ for 14 days) delivered above the SN via osmotic pump enhances striatal DA turnover and decreases nigral DA turnover in amphetamine treated rats (Altar et al., 1992; Martin-Iverson et al., 1994). These neurochemical changes are accompanied by behavioral alterations with BDNF administration causing body weight loss, contralateral rotation following amphetamine administration, and increased locomotor activity (Altar et al., 1992; Martin-Iverson et al., 1994). The *in vivo* effects of BDNF on dopaminergic cell function are further demonstrated by the finding that BDNF (2 $\mu\text{g}/\text{d}$) enhances the behavioral recovery associated with VM cells grafted into 6-OHDA lesioned rats (Sauer et al., 1993). Moreover,

chronic administration of BDNF (12 µg/d for 14 days) above the SNc enhances the firing rate and number of electrically active dopaminergic neurons (Shen et al., 1994). The localization of mRNA for BDNF and its receptor, TrkB, to the SN in adult brain suggests that BDNF may serve to maintain SNc neuronal function in the intact brain, perhaps via an autocrine or paracrine manner (Altar et al., 1994c; Gall et al., 1992; Hyman et al., 1994; Merlio et al., 1992; Seroogy et al., 1994). In addition, exogenous BDNF delivered to the striatum may act on SNc dopaminergic neurons via receptor mediated retrograde transport (Mufson et al., 1994).

Of relevance to the neurodegenerative processes characteristic of Parkinson's disease, pretreatment of VM cells with BDNF protects against the neurotoxic effects of MPP⁺, the active metabolite of MPTP, and 6-OHDA *in vitro*, perhaps by increasing levels of the antioxidant enzyme glutathione reductase (Beck et al., 1992; Hyman et al., 1991; Spina et al., 1992). However, BDNF has shown variable efficacy in protection in *in vivo* lesion paradigms. In rats with partial lesions of the nigrostriatal pathway induced by striatal infusion of 6-OHDA, concomitant supranigral administration of BDNF enhances striatal DA metabolism and reverses lesion induced rotational asymmetry (Altar et al., 1994b). However, BDNF does not alter SN DA levels nor protect against the loss of striatal dopaminergic nerve terminals in this partial lesion paradigm. Initial studies using BDNF failed to show protection of dopaminergic neurons in the SNc following axotomy of the medial forebrain bundle in rat (Knusel et al., 1992; Lapchak et al., 1993). However, a more recent preliminary study suggests that BDNF is neuroprotective in the axotomy paradigm (Hagg, 1994). We have recently demonstrated that fibroblasts genetically engineered to secrete BDNF

protect against SNc neuronal degeneration induced by the mitochondrial complex I inhibitor MPP⁺ (Frim et al., 1994a). In this *in vivo* model of nigral cell degeneration, striatal infusion of MPP⁺ results in a loss of 50-75% of Nissl-stained neurons and approximately 65% of TH neurons in the SNc (Frim et al., 1994a; Srivastava et al., 1993), presumably via retrograde transport (Campbell et al., 1990) and subsequent inhibition of complex I of the mitochondrial electron transport chain (Nicklas et al., 1987). Implantation of BDNF-secreting fibroblasts in the mesencephalon of adult rats 7 days prior to lesioning attenuated the SNc dopaminergic cell loss caused by subsequent administration of MPP⁺ (Frim et al., 1994a). In the present study we evaluated the effects of genetically engineered fibroblasts releasing BDNF on DA content and turnover in the SNc in the MPP⁺ model of nigral degeneration and show that these transfected fibroblasts can mediate a neuroprotective effect as well as an enhancement of DA levels in the SNc.

Materials and methods

Construction of BDNF-secreting fibroblast cell line. An immortalized fibroblast cell line (Rat-I, initially provided by M. Rosenberg, University of California, San Diego) was genetically engineered to secrete BDNF by infection with a retrovirus vector containing a human cDNA encoding a full-length preproBDNF precursor (provided by G. Walz and B. Seed, Massachusetts General Hospital) as previously described (Frim et al., 1993a; Frim et al., 1994a). The BDNF transgene was under the control of the Moloney murine leukemia virus long terminal repeat promoter in a construct containing a neomycin

resistance gene driven by an internal Rous sarcoma virus promoter. Transgenic clones were selected under the neomycin analog G418 and further characterized by an *in vitro* rat dorsal root ganglion bioassay and fetal VM culture assay (Frim et al., 1994a).

Northern blot analysis. BDNF mRNA production by the transfected cell line was assessed by Northern blotting. Total RNA was extracted from cell pellets by guanidinium thiocyanate/cesium chloride centrifugation (Chirgwin et al., 1979), resolved by electrophoresis in 1.4 % agarose gels containing 2.2 M formaldehyde (Lehrach et al., 1977), and transferred to nylon membranes (Hybond, Amersham). Blots were hybridized overnight to a ^{32}P -labeled cRNA probe, washed, and autoradiographed as described (Frim et al., 1988). The preproBDNF cRNA probe was synthesized after placement of the full-length human preproBDNF cDNA into the polylinker site of plasmid Bluescript-SK (Stratagene), linearization of the plasmid with HindIII, and transcription with T3 RNA polymerase in the presence of radioactive nucleotide, per product instructions.

Enzyme linked immunoassay. In order to determine the level of BDNF secretion by the control and transfected cells, BDNF levels were quantified in four 24 hour samples of conditioned media collected from confluent plates of BDNF[-] and BDNF[+] cell cultures grown in Dulbecco's medium supplemented with 10% fetal calf serum. BDNF[+] cells were grown in the presence of 0.1% G418. A sensitive and specific enzyme-linked immunoassay (EIA) detected mature BDNF protein and did not recognize NT-3, NT-4/5, or NGF at

concentrations up to 100 ng/ml (Altar et al., 1995). The EIA employed a BDNF-specific monoclonal antibody of the IgG1 isotype as a capture antibody and a biotinylated, affinity-purified rabbit anti-BDNF polyclonal antibody as a reporter system. The BDNF EIA was used to measure BDNF levels in 100 μ l aliquots of the culture media. The optical density (OD) produced by the colorimetric reaction of each sample was read on a Thermomax plate reader (Molecular Dynamics, Menlo Park, CA) at 450 nm with 570 nm correction for plastic interference. Data were analyzed with the Softmax 881 Software package (Molecular Dynamics). The lowest concentration of the BDNF standard which produced an OD value of twice the mean OD value obtained for dilution buffer only was 10 pg/ml, and a linear range of 10 pg/ml to 1.25 ng/ml was routinely obtained with the EIA.

Cell preparation, surgical implantation, and MPP+ lesion procedures.

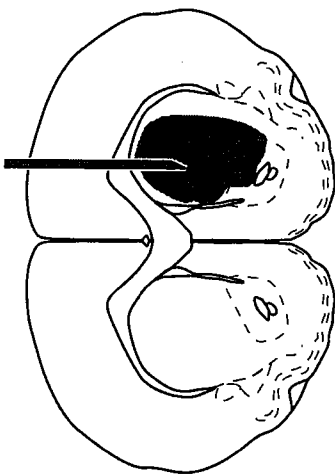
Cells were harvested with trypsin, washed and resuspended in 0.1 M PBS, pH 7.4, with 1.0 μ g/ml CaCl_2 , 1.0 μ g/ml MgCl_2 , and 0.1% glucose at a concentration of 1×10^5 cells/ μ l. Cell number and viability were assessed by trypan blue dye exclusion prior to implantation.

Adult male Sprague-Dawley rats (Charles River Laboratories) were anesthetized with pentobarbital (65 mg/kg, ip) and placed in a Kopf stereotaxic apparatus. BDNF-secreting Rat-I cells (BDNF[+], n=8) or control Rat-I cells (BDNF[-], n=8) were implanted into the dorsal tegmentum of the mesencephalon (coordinates relative to bregma or dura: AP: -5.3 mm, L: -2.4 mm, V: -6.0 mm) (Figure 9). Using a 10 μ l Hamilton syringe, a total of 2×10^5 cells were infused in a volume of 2 μ l over 2 minutes followed by a 2 minute pause prior to withdrawal

Figure 9. A. Schematic diagram of coronal rat brain section indicating MPP+ induced striatal lesion area. B. Schematic representation of mesencephalic graft placement and area dissected for neurochemical analyses. C. Cresyl violet stained section through striatum of MPP+ lesioned rat. D. TH immunostained section through the mesencephalon of lesioned, BDNF[-] rat. Note loss of TH-IR neurons in the SNc ipsilateral to the lesion relative to the intact side. E. TH immunostained section through the mesencephalon of lesioned, BDNF[+] rat. Note neuroprotective effect of BDNF as indicated by the increased cell number in the SNc relative to the BDNF[-] implanted rat. Scale bar in panel C = 500 μ m, panels C and D = 1 mm.

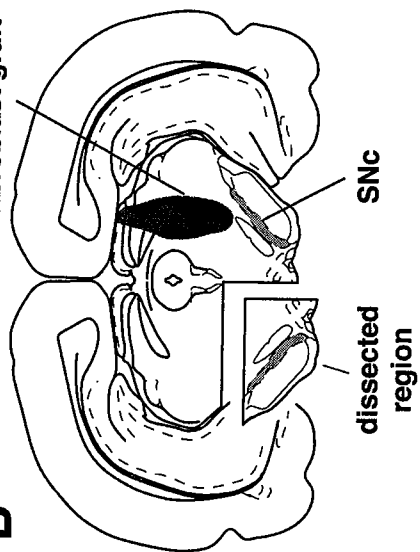
MPP+ injection
into striatum

A



BDNF-secreting
fibroblast graft

B



C



D



E

of the needle. One week following cell implantation, all rats were anesthetized as described above and received a 1 μ l striatal infusion of 100 nmol MPP⁺ over one minute (coordinates calculated relative to bregma or dura: AP: +1.0, L:-2.5, V: -4.5) (Figure 9) The needle was withdrawn after an additional one minute.

Neurochemical analysis and histology. Seven days following MPP⁺ lesioning, all rats were sacrificed by decapitation under deep pentobarbital anesthesia, and the brains were rapidly removed. A 3 mm thick coronal section was cut at the level of the mesencephalon, and the right and left SN were dissected on ice as previously described (Isacson et al., 1985) (Figure 9B), placed in 400 μ l chilled 0.1 M perchloric acid, and frozen on dry ice prior to storage at -80 °C. Samples were sonicated and centrifuged, and the supernatants were evaluated for levels of DA and 3,4 dihydroxyphenylacetic acid (DOPAC) by HPLC with electrochemical detection as described (Beal et al., 1990).

In an analogous series of experiments, animals were implanted with BDNF[-] cells (n = 7) or BDNF[+] cells (n = 8), lesioned in the aforementioned manner, and perfused 7 days after lesioning. Serial sections of tissue were either stained with cresyl violet or immunostained for tyrosine hydroxylase as described (Frim et al., 1994a). These histological results have been reported in part previously (Frim et al., 1994a).

Statistical analysis. Comparison of BDNF production by control and transfected cells was expressed as ng BDNF/10⁵ cells/24 hours and analyzed by Student's t-test. Neurochemical data for the left (intact) and right

(grafted/lesioned) SN were analyzed by one-way analysis of variance with Fisher post-hoc comparison using Statview 4.0 (Abacus).

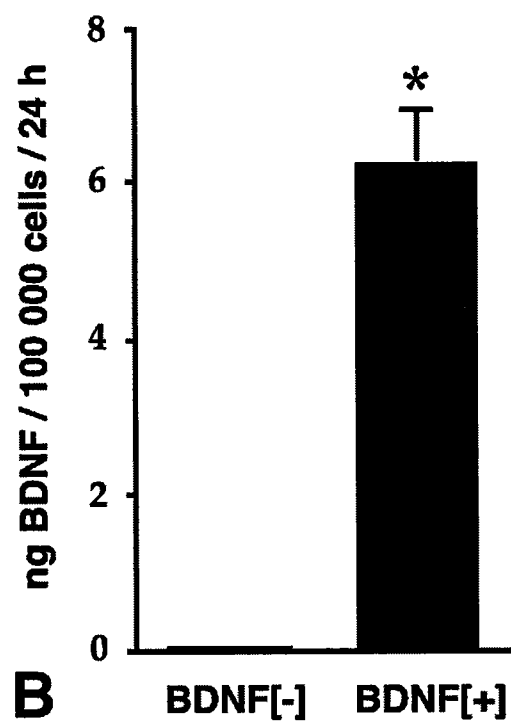
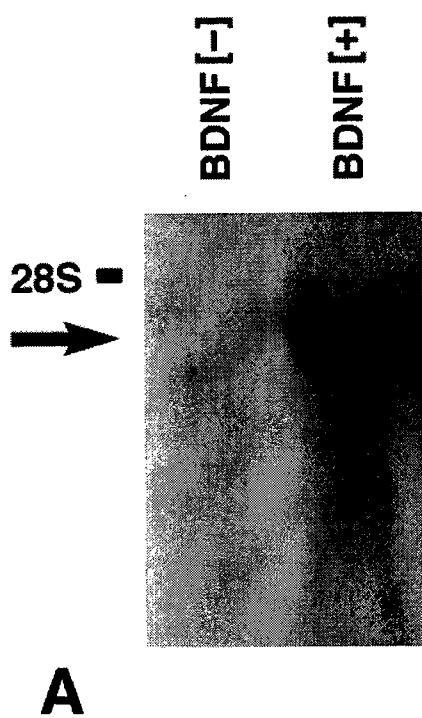
Results

BDNF mRNA expression and BDNF secretion by genetically engineered fibroblasts. Transgenic BDNF mRNA synthesis was confirmed by Northern blotting of total RNA from BDNF[+] cells followed by hybridization to a radiolabeled human preproBDNF antisense cRNA probe. This analysis revealed a single band representing an mRNA species of approximately 5.1 kB in size (Figure 10A, Lane BDNF[+]), consistent with the transgenic construct of preproBDNF-neoR, and of similar size to previously described preproNGF-neoR mRNA transcribed in the identical vector backbone (Wolf et al., 1988). Uninfected Rat-I cells (BDNF[-]) did not contain any hybridization signal (Figure 10A, Lane BDNF[-]).

BDNF secretion by the engineered cells was quantified by EIA. The BDNF-transfected cell line produced 6.3 ± 0.7 ng BDNF/ 10^5 cells/24h (mean \pm SEM) whereas the control Rat-I cell line produced 0.070 ± 0.007 ng BDNF/ 10^5 cells/24 h. The secretion rates differed by a factor of 90, and this difference was highly significant ($p = 0.0001$) (Figure 10B).

Effects of striatal MPP+ infusion and BDNF-secreting fibroblasts on SNc. Striatal infusion of MPP+ resulted in a significant lesion and loss of striatal neurons. A section through the striatum of an MPP+ lesioned rat 7 days post-lesion is shown in Figure 9C. This striatal lesion is associated with loss of TH-IR

Figure 10. A. Northern blot analysis of total RNA from BDNF[-] and BDNF[+] cells hybridized to a radiolabeled human BDNF cRNA probe. Note presence of hybridization band in BDNF[+] cell lane of approximately 5.1 kb (arrow). There is no corresponding band in the BDNF[-] cell lane. -, 28S RNA. B. Comparison of BDNF secretion by control (BDNF[-]) and genetically engineered (BDNF[+]) fibroblast cell line. Transfected cells produce BDNF at a level 90-times above that of control cells. (n=4 per group; Error bars represent SEM. * p=0.0001).



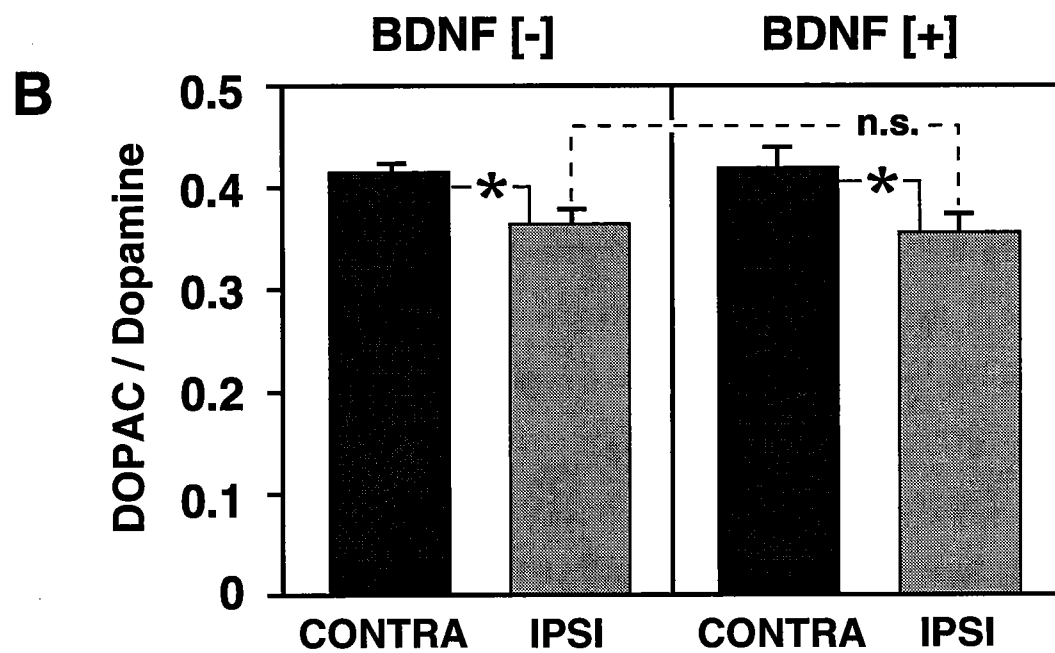
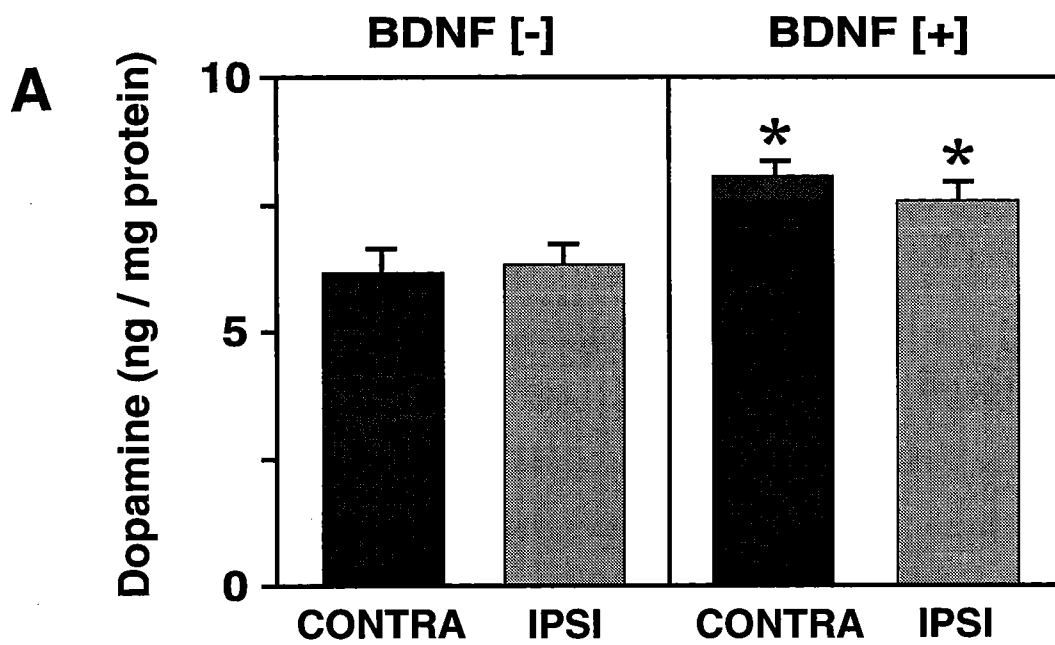
neurons in the SNc which has been quantified in a parallel series of experiments to be a loss of approximately 65% (Frim et al., 1994a). Figure 9 D illustrates this loss of TH-IR neurons in the SNc associated with striatal infusion of MPP+ in a representative rat implanted with BDNF[-] fibroblasts. Supranigral implantation of BDNF[+] fibroblasts attenuated this neuronal loss as illustrated by a representative section through the VM of a rat implanted with BDNF-secreting fibroblasts (Figure 9E).

Effects of BDNF-secreting fibroblasts on SN DA levels and metabolism.

DA and DOPAC levels were evaluated in the left and right SN one week following infusion of MPP+ into the right striatum. There was no side to side difference in SN DA levels in either the BDNF[-] or BDNF[+] implanted groups one week following striatal MPP+ administration (Figure 11A) However, the mean DA level was significantly increased in the BDNF[+] group (7.6 ± 0.4 ng DA/mg protein) as compared to the BDNF[-] group (6.3 ± 0.4) ipsilateral to the lesion. This increase in the BDNF[+] group was also significantly greater than the DA level in the BDNF[-] group contralateral to the lesion (6.2 ± 0.5), indicating a supra-normal DA content associated with BDNF delivery. In addition, on the side contralateral to the implant, DA levels were significantly increased in the BDNF[+] group (8.1 ± 0.3) as compared to the BDNF[-] group.

DA turnover, as measured by DOPAC/DA ratios, was significantly decreased in both the BDNF[-] and BDNF[+] groups on the side ipsilateral to the lesion (BDNF [-] = 0.36 ± 0.02 ; BDNF[+] = 0.36 ± 0.02) relative to the corresponding intact contralateral side (BDNF [-] = 0.42 ± 0.01 ; BDNF[+] = 0.42 ± 0.02) (Figure 11B). There was no significant difference when comparing the

Figure 11. A. Substantia nigra DA concentration in BDNF[-] and BDNF[+] groups ipsilateral (IPSI) and contralateral (CONTRA) to graft and lesion. Mean \pm SEM; * $p < 0.05$ compared to BDNF[-] CONTRA and IPSI. B. DOPAC/DA ratios for BDNF[-] and BDNF[+] groups on the ipsilateral or contralateral sides, respectively. Mean \pm SEM; * $p < 0.05$; n.s. = non-significant.



DOPAC/DA ratios from the ipsilateral or contralateral side in the BDNF[+] group with the respective side in the BDNF[-] group demonstrating that DA turnover is independent of BDNF treatment. (Figure 11B).

Discussion

The present study demonstrates that supranigral, cell-mediated delivery of BDNF is able to enhance SN DA concentration in a rat MPP+ model of Parkinson's disease. In a previous study, we found histologically that BDNF is able to protect dopaminergic neurodegeneration associated with striatal infusion of MPP+ *in vivo* (Frim et al., 1994a). Of note is that these effects are mediated by a level of BDNF that is substantially lower than that employed in other *in vivo* studies of dopaminergic neuronal function and nigral degeneration.

Striatal infusion of MPP+ is associated with dopaminergic cell loss in the SNc due to retrograde transport (Campbell et al., 1990; Frim et al., 1994a; Srivastava et al., 1993). In previous histological studies using this MPP+ model of nigral degeneration, we have shown a loss of approximately 65% of TH-IR neurons in the SNc (Frim et al., 1994a; Srivastava et al., 1993). Yet, in the present study, we found that this decrease in cell number was not associated with a concomitant decrease in SN DA levels in either the BDNF[+] or BDNF[-] group one week following MPP+ administration. This maintenance of DA levels suggests a compensatory increase in DA synthesis by the surviving cells and/or decreased turnover or decreased release by cells metabolically compromised by MPP+. Our finding of decreased DOPAC/DA ratios on the side ipsilateral to the lesion in both the BDNF[+] and BDNF[-] groups indicates that striatal infusion of

MPP+ decreases DA turnover in the remaining SN dopaminergic neurons and thus may contribute to the apparent maintenance of DA levels in the lesioned SN. Moreover, as MPP+ infused into the striatum is not retrogradely transported to the VTA (Campbell et al., 1990) thus sparing the neurons in this area, it is possible that the dopaminergic neurons of this region contained in the dissected area (see Figure 9B) may contribute to the observed maintenance of DA levels. Furthermore, it is possible that some dopaminergic neurons in the SNc remain alive yet are not detected by TH immunostaining. Such a loss of neurotransmitter expression is associated with damage to cholinergic neurons (Hagg et al., 1988; Lams et al., 1988). However, this scenario is less likely in the MPP+ paradigm as Nissl staining reveals a similar loss of neurons compared to TH (Frim et al., 1994a). Moreover, at 5 months following the striatal infusion of MPP+, there is no reversal of lesion effects (Srivastava et al., 1993). In addition, one cannot rule out the possibility that the control BDNF[-] fibroblasts may have some positive effect on maintaining DA levels. Nevertheless, the significantly higher DA levels in the BDNF[+] group relative to the BDNF[-] group ipsilateral to the lesion neurochemically support our previous histological evidence for BDNF mediated dopaminergic neurotrophism.

The increase in DA ipsilateral to the lesion associated with BDNF delivery was also greater than the DA level in the BDNF[-] group contralateral to the lesion indicating a supra-normal DA content associated with BDNF. Furthermore, the finding of elevated DA levels in the BDNF[+] group relative to the BDNF[-] group on the side contralateral to the lesion demonstrates that the implantation of the BDNF-secreting graft has distant effects on the contralateral SN. This distant effect is unexpected in view of previous data indicating that

exogenous BDNF delivered via pump does not diffuse further than approximately 1.5 mm in brain parenchyma (Morse et al., 1993) and suggests that cell-mediated delivery may facilitate distant effects, perhaps via an association of BDNF with membrane derived molecules that aid diffusion or by combinatorial trophic effects with other cell-derived factors (Kennedy et al., 1994; Serafini et al., 1994; Takayama et al., 1995). Indeed, in the quinolinic acid lesion model of Huntington's disease, NGF delivered by fibroblasts (Frim et al., 1993d; Schumacher et al., 1991) or by polymer-encapsulated baby hamster kidney cells (Emerich et al., 1994) implanted prior to lesioning shows a greater protective effect than does NGF delivered via intracranial cannulae at the time of lesioning (Venero et al., 1994). Moreover, consistent with our data, other neurotrophic factors such as GDNF have been shown to mediate effects in the contralateral SN by diffusion from the injection site (Tomic et al., 1995a). With respect to the metabolic effects evaluated in this study, it is noteworthy that while BDNF does increase DA levels, it does not alter relative DA turnover as evidenced by the finding that DOPAC/DA ratios did not differ between groups on the right or left sides.

The enhancement of DA levels in the SN with supranigral delivery of BDNF may be related to the electrophysiological finding that BDNF enhances dopaminergic neuronal firing in the SNc (Shen et al., 1994). These functional effects are presumably mediated by the interaction of BDNF with its high-affinity receptor, TrkB, which has been localized to neurons of the adult rat SN (Hyman et al., 1994; Merlio et al., 1992). Functional enhancement of dopaminergic neurons has been demonstrated in association with striatal and supranigral BDNF infusions (Altar et al., 1994b; Altar et al., 1992; Martin-Iverson et al., 1994).

In contrast, chronic delivery of BDNF has also been suggested to decrease striatal dopaminergic cell function (Lapchak et al., 1993). However, damage to the SNc associated with repeated injections may account for this apparently contradictory finding thereby illustrating the importance of administration route. The neuroprotection mediated by BDNF-secreting fibroblasts in the MPP+ rat model of nigral degeneration (Frim et al., 1994a) is in contrast to other reports in which BDNF failed to protect against dopaminergic neuronal loss (Knusel et al., 1992; Lapchak et al., 1993). In addition to variations in lesion methodologies as well as routes of administration and duration of trophic factor delivery, the dose of BDNF delivered may be of particular significance in order to achieve optimal neuroprotection. Previous neuroprotection studies have delivered between 1 and 4.5 micrograms daily or every second day via intraparenchymal injections whereas the fibroblasts implanted in the present study secrete approximately 12 ng per day thereby suggesting a dose-response curve in which the optimal neuroprotective dose *in vivo* may fall lower than has previously been tested. Indeed such a dose-response effect has been reported *in vitro* for the trophic effects of NT-3 and NT-4 on dopaminergic neurons (Hyman et al., 1994), and studies of axotomized SN neurons as well as axotomized motor neuron rescue demonstrate a submaximal response with higher doses of BDNF (Hagg, 1994; Vejsada et al., 1994). As suggested by Vejsada, et al. (1994), high levels of ligand may result in decreased affinity or down-regulation of the high affinity TrkB receptor or an upregulation of the inactive truncated TrkB receptor, thereby accounting for the decreased efficacy of higher doses of BDNF.

The results of the present study neurochemically confirm BDNF-mediated protection of dopaminergic neurons following mitochondrial blockade associated

with MPP+ and furthermore indicate an enhancement of DA levels caused by delivery of BDNF by genetically engineered fibroblasts. Of particular significance is the efficacy of the comparatively low level of BDNF delivery in this paradigm. The neurotrophic effects of BDNF on dopaminergic neurons *in vivo* indicate the potential relevance of BDNF to disorders characterized by neuronal degeneration and DA imbalance.

ACKNOWLEDGEMENT

All studies presented in this chapter were conducted by the candidate with the exception of the BDNF EIA which was performed by Regeneron Pharmaceuticals and the neurochemical analysis which was done in collaboration with Dr. Flint Beal's laboratory, Neurology Service, Massachusetts General Hospital. This material has been published as:

Galpern, W. R., Frim, D. M., Tatter, S. B., Altar, C. A., Beal, M. F., and Isacson, O. (1996). Cell-mediated delivery of brain-derived neurotrophic factor enhances dopamine levels in an MPP+ rat model of substantia nigra degeneration. *Cell Transplantation* 5, 225-232.

CHAPTER IV

ANTIOXIDATIVE MECHANISM OF NEUROTROPHIC FACTOR-MEDIATED NEUROPROTECTION AGAINST MITOCHONDRIAL TOXICITY

Abstract

NGF-secreting fibroblasts are able to protect against the striatal neurodegeneration induced by the mitochondrial toxin 3-nitropropionic acid (3-NP). In the present study, we have investigated whether the neuroprotective effects of NGF are mediated through antioxidative mechanisms. Rats were grafted in the corpus callosum with NGF[+] or NGF[-] fibroblasts 7 days prior to the administration of 3-NP. The generation of peroxynitrite was evaluated by measuring the striatal levels of 3-nitrotyrosine. NGF grafts significantly decreased the 3-NP induced generation of 3-nitrotyrosine, presumably by decreasing peroxynitrite formation. These findings suggest that NGF may protect against neuronal death by inhibiting the production of nitric oxide or decreasing the levels of superoxide radicals, thereby decreasing the generation of oxidative agents such as peroxynitrite.

Introduction

HD is an autosomal dominant disorder caused by expansion of a CAG repeat within the IT15 gene (The Huntington's Disease Collaborative Research Group, 1993). This disorder is characterized clinically by choreiform movements and cognitive impairment (Martin and Gusella, 1986) and neuropathologically by progressive degeneration of striatal spiny projection neurons with relative sparing of striatal NADPH-diaphorase and acetylcholinesterase aspiny interneurons as well as afferent fibers and fibers of passage (Dawbarn et al., 1985; Ferrante et al., 1987; Ferrante et al., 1985; Graveland et al., 1985; Kowall et al., 1987; Reiner et al., 1988). Paralleling the loss of spiny neurons is a significant decrease in striatal glutamic acid decarboxylase and GABA as well as a decrease in choline acetyltransferase (Bird et al., 1973; Perry et al., 1973).

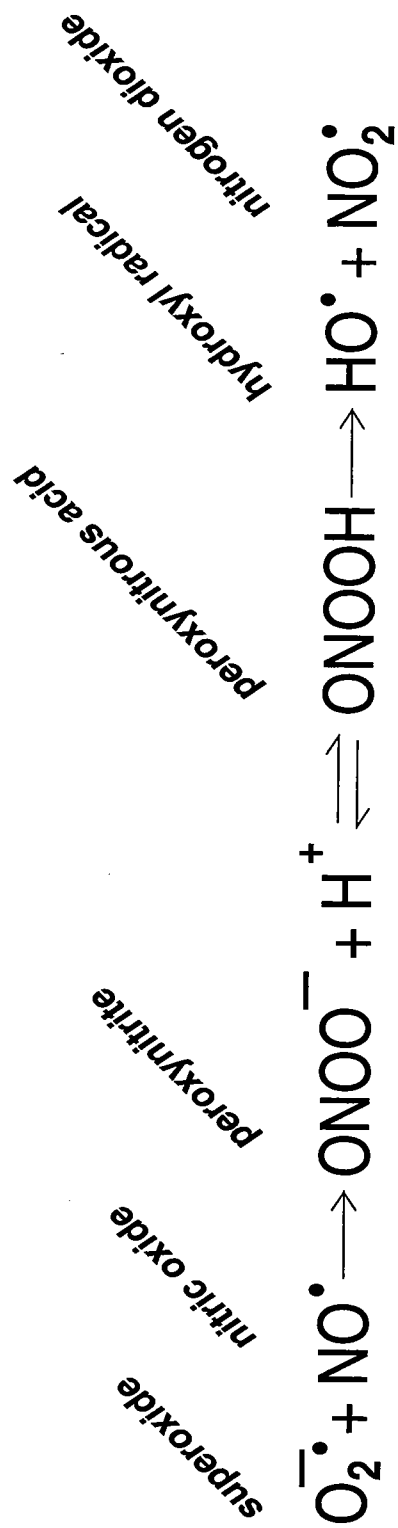
Although the genetic abnormality underlying HD has been identified, the precise process of neuronal death remains unknown. Several lines of evidence suggest that the striatal neurodegeneration may be due to excitotoxicity and/or oxidative stress. Indeed, impairments of mitochondrial function (Brennan et al., 1985; Mann et al., 1990; Parker et al., 1990) and energy metabolism (Jenkins et al., 1993) as well as a preferential loss of glutaminoceptive neurons with NMDA receptors (Greenamyre et al., 1985; Young et al., 1988) and reductions in calbindin-D gene expression (Iacopino and Christakos, 1990) are found in HD. While the precise cellular events which cause neuronal death are uncertain, increased intracellular calcium is known to activate numerous deleterious enzyme cascades resulting in the production of superoxide radicals and nitric oxide. Peroxynitrite, formed by the reaction of superoxide and nitric oxide, is a

highly reactive oxidant (Beckman et al., 1990) and may contribute to the neurodegenerative processes in HD (Figure 12).

Administration of the succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NP) produces striatal lesions and behavioral abnormalities characteristic of HD (Beal et al., 1993; Brouillet et al., 1995). 3-NP results in increased generation of hydroxyl radicals and 3-nitrotyrosine (Beal et al., 1995; Schulz et al., 1995a), the product of peroxynitrite mediated nitration of tyrosine (Ischiropoulos et al., 1992). The elevations in hydroxyl radicals and 3-nitrotyrosine formation as well striatal lesion size are attenuated in copper/zinc superoxide dismutase transgenic mice, suggesting a decrease in 3-NP induced peroxynitrite formation due to increased superoxide scavenging (Beal et al., 1995). Furthermore, as inhibition of neuronal nitric oxide synthase (nNOS) by 7-nitroindazole (7-NI) protects against striatal lesions and reduces the increase in 3-nitrotyrosine associated with 3-NP administration, involvement of nitric oxide in 3-NP neurotoxicity is likely (Schulz et al., 1995a).

We have previously shown that NGF secreting fibroblasts implanted in the corpus callosum are also able to protect against subsequent 3-NP induced striatal degeneration ipsilateral to the graft (Frim et al., 1993c). In the present study, the mechanism of this NGF mediated neuroprotection was investigated by evaluating the ability of NGF to protect against the formation of 3-nitrotyrosine associated with 3-NP administration.

Figure 12. Formation of peroxynitrite. Peroxynitrite is formed by the reaction of superoxide and nitric oxide. Systemic administration of 3-NP results in increased generation of 3-nitrotyrosine, the product of peroxynitrite mediated tyrosine nitration.



Materials and methods

Cell preparation, surgical implantation, and 3-NP lesion procedures.

NGF-secreting fibroblasts (Schumacher et al., 1991) were harvested with trypsin, washed, and resuspended in 0.1 M PBS, pH 7.4, with 1.0 $\mu\text{g}/\text{ml}$ CaCl_2 , 1.0 $\mu\text{g}/\text{ml}$ MgCl_2 , and 0.1% glucose at a concentration of 1×10^5 cells/ μl . Cell number and viability were assessed by ethidium bromide-acridine orange prior to implantation.

Young adult male Sprague-Dawley rats (125-150 g, Charles River Laboratories) were anesthetized with pentobarbital (65 mg/kg, ip) and placed in a Kopf stereotaxic apparatus. NGF-secreting Rat-I cells (NGF[+], $n=12$; 177.6 pg NGF/ 10^5 cells/h) or control Rat-I cells (NGF[-], $n=11$; 6.7 pg NGF/ 10^5 cells/h) were implanted into the corpus callosum (coordinates relative to bregma or dura: AP: +1.6 mm, L: -1.4 mm, V: -4.0). Using a 10 μl Hamilton syringe, a total of 1×10^6 cells were infused in a volume of 10 μl over 5 minutes followed by a 5 minute pause prior to withdrawal of the needle. One week following cell implantation, rats received 3 injections of 3-NP (20 mg/kg, ip, pH 7.4; Aldrich Chemicals, Milwaukee, WI) at 12 hour intervals. This dosing regimen has previously been shown to increase the production of 3-nitrotyrosine (Schulz et al., 1995a). For histological analysis of striatal neuronal loss associated with 3-NP administration, an additional group of non-implanted rats ($n = 5$) was injected with 3-NP as described above.

Histological analysis. Two and one-half hours after the final 3-NP injection, the non-grafted 3-NP treated animals were terminally anesthetized

with sodium pentobarbital and perfused through the left ventricle with 250 ml cold heparinized saline (1000 units heparin/ liter 0.9% saline) followed by 250 ml cold 4% paraformaldehyde in 100 mM PBS (pH 7.4). Brains were removed and post-fixed for 8 hours in this same paraformaldehyde solution before being transferred to 30% sucrose in PBS (pH 7.4). Following equilibration, series of 40 μ m coronal sections were cut using a freezing microtome and collected in PBS. Every fourth section was immunostained for the striatal marker dopamine- and c-AMP-regulated phosphoprotein 32 KDa (DARPP-32) in order to evaluate the effects of 3-NP on neuronal survival in the striatum. Free-floating sections were pretreated with 50% methanol and 0.3% hydrogen peroxide in PBS for 20 minutes, rinsed three times in PBS, and then pre-incubated for 1 hour in 10% normal goat serum (NGS) in PBS prior to overnight incubation with DARPP-32 antibody (kindly provided by Dr. Paul Greengard) diluted 1:20,000 in PBS. Sections were then washed in PBS and incubated with goat anti-mouse biotinylated secondary antibody (Sigma) diluted 1:200 in 2% NGS in PBS for 90 minutes. After three PBS rinses, the sections were incubated with avidin-biotin complex (Vectastain ABC Kit ELITE, Vector Labs) for 90 min, rinsed once in PBS and twice in 0.05 M TBS, then developed with 0.04% hydrogen peroxide and 0.05% 3,3'-diaminobenzidine (Sigma) in TBS for 5-15 min.

Neurochemical analysis. Animals were sacrificed by decapitation 2.5 hours after the final 3-NP injection (Schulz et al., 1995a), and the brains were rapidly removed. The right and left striata were dissected on ice from a 2 mm coronal section, placed in 500 μ l chilled 0.1 M perchloric acid, and frozen on dry ice prior to storage at -80 °C. Samples were sonicated and centrifuged, and the supernatants were evaluated for levels of tyrosine and 3-nitrotyrosine by HPLC

with electrochemical detection as previously described (Beal et al., 1990). Tyrosine and 3-nitrotyrosine were measured as described (Schulz et al., 1995a) and expressed as the ratio of 3-nitrotyrosine to tyrosine to adjust for differing concentrations of brain tyrosine.

Statistical analysis. Data were expressed as a ratio of grafted side to non-grafted side for each animal. Mean ratios were determined for the NGF[+] and NGF[-] groups and analyzed by t-test using Statview 4.0 (Abacus).

Results

Sections through the striatum were immunostained for DARPP-32 in order to evaluate the effects of 3-NP on neuronal survival. At 2.5 hours following the final 3-NP dose, there was no evidence of striatal cell loss evident on DARPP-32 staining (Figure 13). Distinct neuronal cell bodies were evident throughout the striatum at this time point, indicating neuronal survival at the time of 3-nitrotyrosine evaluation.

The ratio of 3-nitrotyrosine to tyrosine was evaluated for the left and right striata for all animals in the NGF[-] and NGF[+] groups and expressed as the grafted side relative to the non-grafted side (Fig. 2). This ratio of 3-nitrotyrosine to tyrosine was significantly reduced in the NGF[+] group (0.87 ± 0.14) relative to the NGF[-] group (1.28 ± 0.14 , $p =$; Figure 14), a decrease of 32%.

Figure 13. DARPP-32 immunostained section through the striatum 2.5 hours following the third 3-NP injection. A. Note absence of 3-NP induced striatal lesion at this time point. B. High power view of section shown in A. Cell bodies are clearly distinguishable. Scale bar = 1 mm (A), 100 μ m (B)

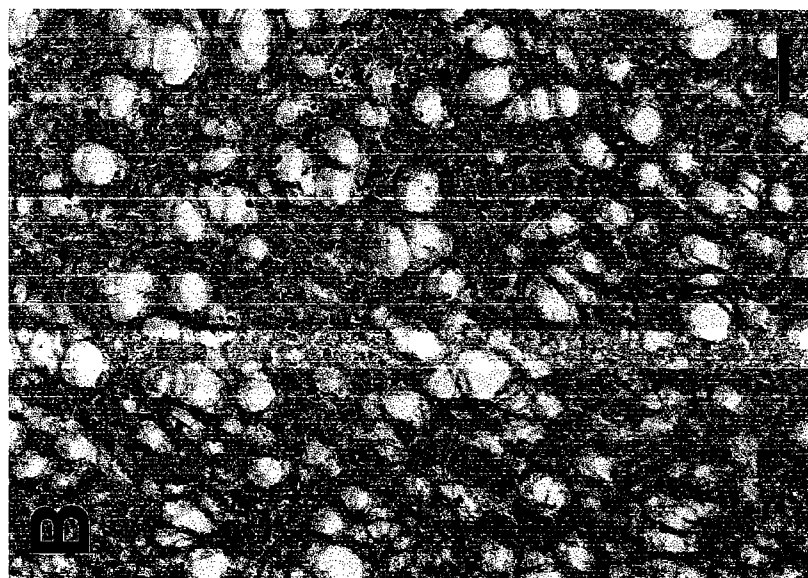
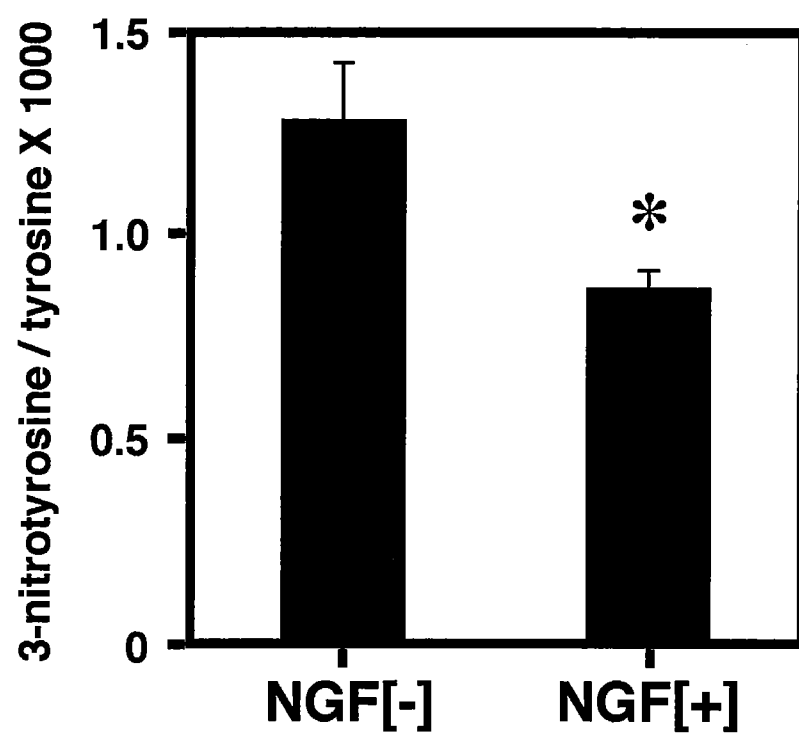


Figure 14. Effect of NGF-secreting fibroblast graft on 3-NP induced generation of 3-nitrotyrosine *in vivo*. The ratio of 3-nitrotyrosine to tyrosine was evaluated for the left and right striata for the 3-NP treated animals in the NGF[+] and NGF[-] groups and expressed as the grafted side relative to the non-grafted side. The ratio of 3-nitrotyrosine to tyrosine was significantly reduced in the NGF[+] group relative to the NGF[-] group, a decrease of 32%. Error bars represent SEM. * $p < 0.05$.



Discussion

The present results demonstrate that NGF-secreting fibroblasts implanted into the corpus callosum, as compared to control fibroblasts, are able to attenuate the production of 3-nitrotyrosine induced by administration of the mitochondrial toxin 3-NP. 3-nitrotyrosine is formed by peroxynitrite-mediated tyrosine nitration, and the reduced production of this marker in NGF[+] grafted rats suggests that the neuroprotective effect of NGF may be mediated in part by reduced peroxynitrite formation. Furthermore, as the reaction of superoxide and nitric oxide generates peroxynitrite, NGF may increase the scavenging of superoxide radicals and/or decrease the production of nitric oxide, possibly by inhibiting nNOS. As shown by the histological results, there is no visible neuronal damage at the time of neurochemical analysis (2.5 hours after 3-NP dosing).

Administration of 3-NP results in the production of hydroxyl radicals and peroxynitrite as well as striatal neurodegeneration (Beal et al., 1995; Schulz et al., 1995a). The precise cellular mechanisms involved in this cell death are not fully understood. As 3-NP induced striatal lesions are prevented by the prior removal of cortical-striatal glutamatergic input via decortication, it is likely that the observed cell death is a consequence of excitotoxicity secondary to impaired energy metabolism (Beal et al., 1993). Moreover, the findings that the hydroxyl radical production and 3-nitrotyrosine formation associated with 3-NP administration are decreased in mice overexpressing SOD (Beal et al., 1995) and in rats treated with the nNOS inhibitor 7-NI (Schulz et al., 1995a) indicate a role for both superoxide and nitric oxide as well as peroxynitrite in 3-NP mediated

neuronal death. Likewise, the present finding of reduced levels of 3-nitrotyrosine in NGF[+] rats as compared to the NGF[-] rats suggests that the neuroprotective effects of NGF may involve a reduction in peroxynitrite formation.

NGF is able to protect against a variety of cellular insults including quinolinic acid (Schumacher et al., 1991) and 3-NP (Frim et al., 1993c) excitotoxicity as well as atrophy and death of cholinergic neurons associated with aging (Fischer et al., 1987) or lesioning (Williams et al., 1986). While the precise neuroprotective mechanisms remain unclear, evidence suggests that NGF may increase the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase (Frim et al., 1994b; Nisticò et al., 1992) as well as stabilize cellular Na⁺/K⁺ ATPases (Varon and Skaper, 1983). Interestingly, while inhibition of nNOS with 7-NI protects against 3-NP toxicity (Schulz et al., 1995a), it fails to protect against the excitotoxic neuronal death associated with quinolinic acid suggesting that the toxicity of quinolinic acid is mediated via an NO-independent mechanism (MacKenzie et al., 1995). As NGF is able to protect against both of these excitotoxins, multiple neuroprotective mechanisms may be induced by NGF.

Impaired energy metabolism, free radicals, and excitotoxicity are likely involved in the pathogenesis of neurodegenerative disorders such as Huntington's disease (Beal, 1995). Experimental evidence suggests that mitochondrial impairment results in secondary excitotoxic cell death (Novelli et al., 1988; Simpson and Isacson, 1993), and the neuronal death caused by 3-NP may be attributed to such processes (Beal et al., 1993). The present finding of reduced 3-nitrotyrosine levels in NGF[+] grafted rats suggests that NGF

neuroprotection may be mediated in part by reduced oxidative stress due to decreased peroxynitrite formation. Due to its ability to prevent neuronal death at the level of oxidative stress, NGF may have applications for future therapeutic interventions in neurodegenerative disorders characterized by mitochondrial impairment and oxidative stress such as HD.

Conclusion

As neurodegenerative processes are thought to be mediated by a combination of excitotoxic mechanisms, free radical damage, and energy impairment, factors which act at any of these levels may interfere with the progression to cell death. Neurotrophic factors are able to protect neurons against a variety of cellular insults indicating their potential therapeutic value for the treatment of neurodegenerative disorders (Mattson et al., 1993). 3-NP toxicity is associated with the production of hydroxyl radicals and peroxynitrite activity. NGF-secreting fibroblasts have previously been shown to protect against the striatal lesions associated with 3-NP induced mitochondrial impairment. The reduced formation of 3-nitrotyrosine following 3-NP administration in NGF[+] grafted rats, as compared to NGF[-] rats, suggests that the neuroprotective mechanism of NGF likely involves inhibition of peroxynitrite. The present data suggest that the antioxidative mechanisms underlying NGF mediated neuroprotection may include superoxide scavenging as well as inhibition of nitric oxide formation. As 3-NP toxicity parallels many of the histological and behavioral features of HD, neurotrophic factors such as NGF

may reduce neuronal vulnerability and protect against ensuing neurodegenerative processes.

ACKNOWLEDGEMENT

All studies presented in this chapter were conducted by the candidate with the exception of the neurochemical analysis which was done in collaboration with Dr. Flint Beal's laboratory, Neurology Service, Massachusetts General Hospital. This material has been submitted for publication:

Galpern, W. R., Matthews, R. T., Beal, M. F., and Isacson, O. NGF attenuates 3-nitrotyrosine formation in a 3-NP model of Huntington's disease.

CHAPTER V

XENOTRANSPLANTATION OF PORCINE FETAL VENTRAL MESENCEPHALON IN A RAT MODEL OF PARKINSON'S DISEASE: FUNCTIONAL RECOVERY AND GRAFT MORPHOLOGY

Abstract

Neurotransplantation of human fetal DA neurons is currently being investigated as a therapeutic modality for PD. However, the practical limitations of human fetal transplantation indicate a need for alternative methodologies. Using the 6-OHDA rat model of PD, we transplanted dopaminergic neurons derived from ED 27 porcine fetuses into the denervated striatum of CyA-treated or non-CyA treated rats. Functional recovery was assessed by amphetamine-induced rotation, and graft survival and morphology were analyzed using neuronal and glial immunostaining as well as in situ hybridization with a porcine repeat element DNA probe. A significant, sustained reduction in amphetamine-induced rotational asymmetry was present in the CyA treated rats whereas the non-CyA treated rats showed a transient behavioral recovery. The degree of rotational recovery was highly correlated to the number of surviving transplanted porcine dopaminergic neurons. TH+ neuronal survival and graft volume were significantly greater in the CyA treated group as compared to the non-CyA group. By donor-specific neuronal and glial immunostaining as well as donor-specific DNA labelling, we demonstrate that porcine fetal neuroblasts are

able to survive in the adult brain of immunosuppressed rats, mediate functional recovery, and extensively reinnervate the host striatum. These findings suggest that porcine DA neurons may be a suitable alternative to the use of human fetal tissue in neurotransplantation for PD.

Introduction

Current therapeutic strategies for the treatment of PD include the transplantation of human fetal dopaminergic neurons in order to replace dopaminergic neurotransmission and reconstruct synaptic circuitry. Data from rat models of PD demonstrate that transplanted human neuroblasts are able to reinnervate the host brain (Brundin et al., 1986; Brundin et al., 1988b; Clarke et al., 1988; Strömberg et al., 1986; Wictorin et al., 1992). In addition, recent clinical reports indicate long-term survival and functional recovery associated with the transplantation of human fetal ventral mesencephalic tissue into the caudate and/or putamen of patients with MPTP-induced (Widner et al., 1992) or idiopathic (Freed et al., 1992; Kordower et al., 1995; Lindvall et al., 1994) parkinsonism. While these initial clinical findings are encouraging, the practical and ethical limitations inherent to current human neurotransplantation protocols remain. The survival rate of dopaminergic neurons following transplantation is approximately 5-10%, and as many as 10-15 fetal VM per patient may therefore be required for sufficient survival and reinnervation (Björklund, 1993). Availability of viable human fetal neural tissue is presently a limiting factor, and

the development of alternate methodologies in clinical transplantation is therefore necessary.

One approach to increase the availability of fetal tissue for surgical procedures would be long-term storage of the cells derived from several embryos. Various storage approaches have been investigated including freezing, culturing, and cool storage (4°C) of the harvested tissue. Unfortunately, cryopreservation of VM cells in liquid nitrogen prior to transplantation has yielded variable results in experimental models (Collier et al., 1987; Frodl et al., 1994a; Redmond et al., 1988; Sauer et al., 1992). Similarly, while grafted cultured VM neurons are able to survive (Brundin et al., 1985a; Brundin et al., 1988a) graft size has been shown to be markedly reduced relative to fresh tissue grafts (Brundin et al., 1988a). Interestingly, cool storage of rat or human VM tissue fragments in hibernation medium for up to 5 or 3 days, respectively, prior to dissociation and grafting has been reported not to affect graft survival adversely (Sauer and Brundin, 1991). Indeed, pooled human fetal tissue has been stored in hibernation medium for short intervals (up to 48 hours at 8°C) prior to transplantation in clinical trials (Freeman et al., 1995; Kordower et al., 1995). However, this approach is temporally limited as graft survival is significantly reduced following longer storage periods (Sauer and Brundin, 1991). Yet, graft viability has recently been demonstrated using extended periods of cool storage (up to 8 days) in combination with microtransplantation techniques (Nikkhah et al., 1995) suggesting long-term cool storage may be clinically feasible.

A further approach to circumventing the limitations associated with human fetal neural transplantation is the grafting of fetal neuroblasts derived from a xenogeneic donor. Xenotransplantation allows for the sterile harvesting

of large quantities of pathogen-free tissue of the desired embryonic age. In a variety of experimental paradigms, transplanted fetal xenogeneic neuroblasts survive in and reinnervate the adult host brain (see Pakzaban and Isacson, 1994). It has previously been reported that xenografts containing DA cells derived from ED 21-ED 26 porcine VM are able to survive in the immunosuppressed adult rat (Freeman et al., 1988; Huffaker et al., 1989). In addition, long-term graft survival and reduction of amphetamine-induced rotational asymmetry were found in immunosuppressed rats as compared to non-transplanted and non-neuronal transplant controls (Huffaker et al., 1989).

Alternatively, the constraint of tissue supply could be overcome by grafting other types of DA releasing cells. Catecholamine-releasing cells derived from the adrenal medulla have been investigated as a potential autologous tissue source for the treatment of PD. However, results from these studies have shown poor graft survival and little sustained therapeutic benefit (Goetz et al., 1991). Implantation of cells genetically engineered to release DA may provide an additional approach to replenishing depleted striatal DA levels. Fibroblasts genetically modified to express the gene for TH have been shown to release L-dopa *in vivo* and attenuate apomorphine-induced rotational asymmetry in 6-OHDA lesioned rats (Fisher et al., 1991; Horellou et al., 1990; Wolff et al., 1989). More recently, several groups have attempted to infect striatal cells directly with the TH gene via herpes simplex virus (During et al., 1994), adenovirus (Horellou et al., 1994), or adeno-associated virus (Kaplitt et al., 1994) vectors. Additionally, virus vectors have been used to infect human neural progenitors prior to transplantation (Martinez-Serrano et al., 1995; Sabaté et al., 1995) suggesting the potential utility of *ex vivo* genetic engineering of progenitor cells. While the

approach of genetic modification of cells holds promise for future therapeutic interventions, current methodologies are constrained by limited gene expression and functional recovery (Björklund, 1993; Lindvall, 1995) as well as the cytopathogenicity and the low rate of infectivity (Isacson, 1995) associated with virus vectors. Moreover, the constitutive release of DA by non-neuronal cells or non-dopaminergic neurons may not be as effective in ameliorating parkinsonian symptoms as compared with the regulated, synaptic release by transplanted dopaminergic neurons (Björklund, 1992; Björklund, 1993).

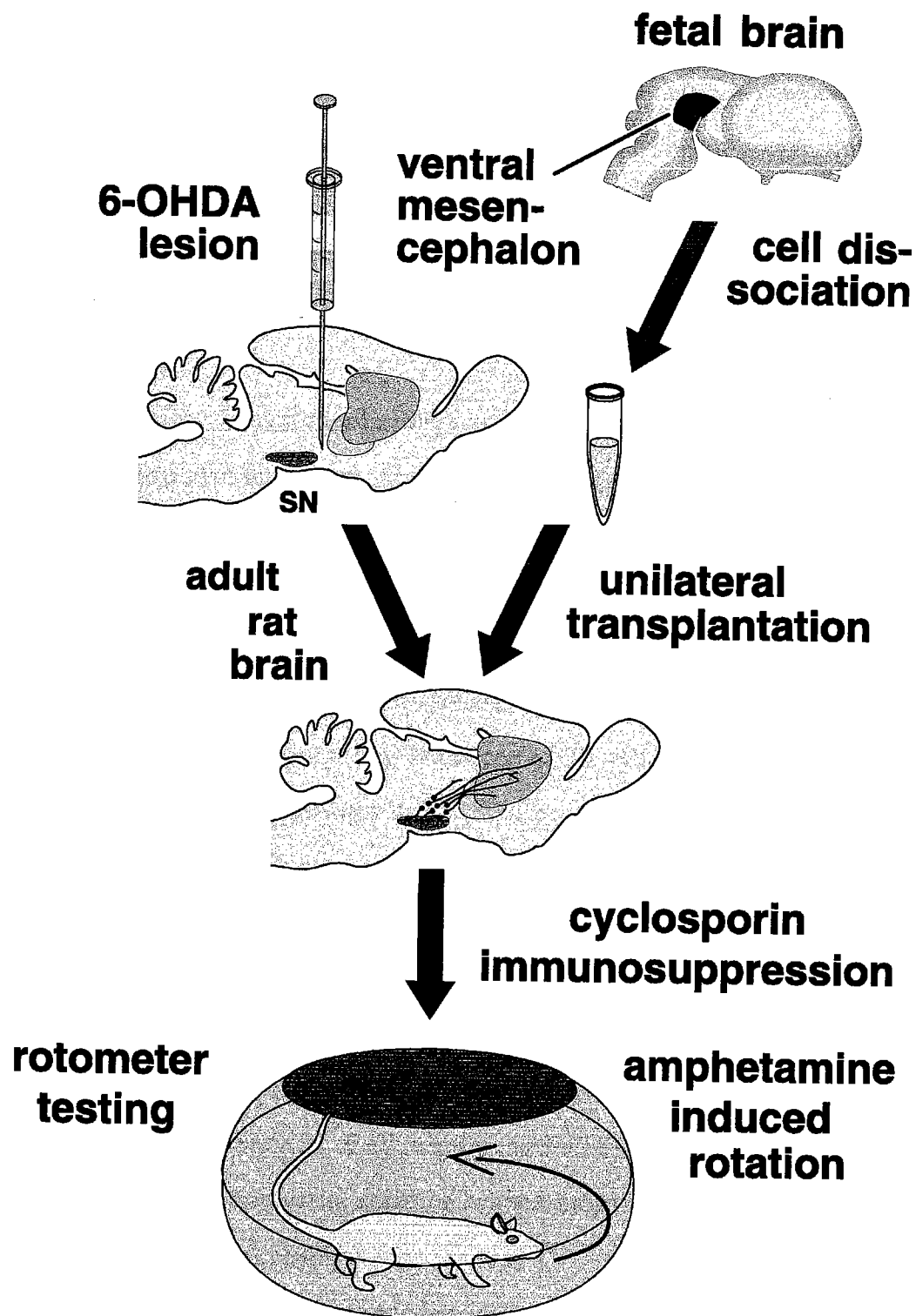
Recognizing the significant constraints of using human fetal tissue for transplantation and the potential limitations of genetically modified cells, we have investigated the ability of porcine neurons to reconstruct neuronal circuitries *in vivo* (Deacon et al., 1994; Isacson et al., 1995). In the present study, using the 6-OHDA rat model of PD, we have transplanted neuroblasts derived from the VM of porcine fetuses (ED 27) into the striatum to further assess the potential of fetal porcine dopaminergic neurons as a donor source for transplantation. Graft survival and amphetamine-induced rotation were evaluated, and the relationship between neuron survival and the associated functional recovery as well as the effects of CyA on these parameters were analyzed. In addition, xenograft morphology and graft-derived striatal reinnervation were investigated using donor-specific glial and neuronal markers as well as in situ hybridization with a porcine repeat element (PRE) DNA probe.

Materials and methods

Lesion surgery, behavioral testing, and experimental groups. Adult female Sprague-Dawley rats received a unilateral DA-depleting lesion by stereotaxic injection of 6-OHDA (3.6 $\mu\text{g}/\mu\text{l}$ 0.02% ascorbic acid in saline) at two sites (2.5 $\mu\text{l}/\text{site}$) in the medial forebrain bundle using a 10 μl Hamilton syringe (coordinates relative to bregma: AP = -4.0, L = -0.8, V = -8.0, incisor bar = +3.4; AP = -4.4, L = -1.2, V = -7.8, incisor bar = -2.4). 6-OHDA was infused at a rate of 1 $\mu\text{l}/\text{min}$, and the needle was left in place for an additional 2 minutes prior to withdrawal. All surgeries were conducted under sodium pentobarbital (65 mg/kg, ip) anesthesia in a Kopf stereotaxic frame. Three weeks post-lesion, rats were tested for amphetamine-induced (5 mg/kg, ip) rotational asymmetry using automated rotometers (San Diego Instruments) (Ungerstedt and Arbuthnott, 1970) (Figure 15).

Twenty-four rats with a net ipsilateral rotation (ipsilateral rotations minus contralateral rotations) greater than 800 rotations per 90 minutes, reflecting an approximate 97% DA depletion (Schmidt et al., 1982), were divided into two experimental xenotransplantation groups balanced with respect to pre-transplant rotation scores. In group A (n = 12), rats received daily injections of CyA (Sandimmune; 10 mg/kg, diluted in olive oil, sc; Sandoz, East Hanover, NJ) commencing one day prior to transplantation. We have previously shown this dose to be effective (Pakzaban et al., 1995). In group B (n = 12), rats received no CyA. CyA treated rats received tetracycline (Panamycin; approximately 20-40 mg/kg/d; Upjohn, Kalamazoo, MI) via their drinking water (250 mg/l) beginning 30 days after transplantation. All rats received preoperative doses of

Figure 15. Schematic diagram of transplantation and behavioral testing methods. A cell suspension of fetal porcine VM was prepared and subsequently implanted unilaterally into the 6-OHDA denervated striatum. At approximately 4 week intervals post-transplantation, rats were tested for amphetamine-induced rotational asymmetry.



the antibacterial agent cephalothin (Keflin; 10 mg/kg, sc; Lilly, Inc., Indianapolis, Indiana) and the anti-inflammatory agent methylprednisolone (Depo-Medrol; 5 mg/kg, im; Upjohn, Kalamazoo, MI). Rotational asymmetry was monitored at 4-6 week intervals following transplantation to assess the graft-associated functional recovery. One rat from Group A died during transplantation surgery and one rat from Group B died following amphetamine rotation.

Preparation and transplantation of porcine fetal ventral

mesencephalon. Fetuses were obtained from ultrasound-confirmed pregnant Yorkshire pigs 27 days post-insemination according to the standard procedures of Tufts University School of Veterinary Medicine (Grafton, MA). Fetuses (CRL = 21 mm) were removed to a dish with cold sterile calcium- and magnesium-free Dulbecco's PBS, and the VM was dissected from the surrounding tissue and collected in a petri dish containing Dulbecco's PBS. The VM fragments were incubated at 37°C for 10 minutes in 1.5 ml of pre-warmed 0.05% Trypsin-0.53 mM EDTA (Sigma) in calcium- and magnesium-free Hanks Balanced Salt Solution (HBSS). The tissue then was washed four times with HBSS with 50 µg/ml Pulmozyme (human recombinant DNase; Genentech, San Francisco, CA), and then gently triturated through a series of fire-polished Pasteur pipettes of decreasing diameter until a cell suspension containing single cells and small clumps of cells was obtained. Cell number and viability were determined under fluorescence microscopy using acridine orange-ethidium bromide as previously described (Brundin et al., 1985b).

Rats were anesthetized as for lesioning and were grafted in the denervated striatum with a suspension of approximately 200,000 VM cells. Using a 10 µl

Hamilton syringe, 2 μ l of the VM cell suspension was injected at each of 2 striatal sites at a rate of 1 μ l /min followed by a 2 minute pause prior to withdrawal of the needle (coordinates relative to bregma: AP = +1.5, L = -2.8, V = -5.0 to -4.5; AP = +0.5, L = -3.0, V = -5.0 to -4.5, incisor bar = -3.3).

Perfusion and histological processing. Nineteen weeks post-transplantation, animals were terminally anesthetized with sodium pentobarbital and perfused through the left ventricle with 250 ml cold heparinized saline (1000 units heparin/ liter 0.9% saline) followed by 250 ml cold 4% paraformaldehyde in 100 mM PBS (pH 7.4). Brains were removed and post-fixed for 8 hours in this same paraformaldehyde solution before being transferred to 30% sucrose in PBS (pH 7.4). Following equilibration, series of 40 μ m coronal sections were cut using a freezing microtome and collected in PBS.

Every sixth section was stained with cresyl violet in order to evaluate graft survival. To assess neuronal survival and graft morphology, adjacent sections were immunostained by the avidin-biotin peroxidase method (Vector Labs, Burlingame, CA) for TH, donor-derived neurofilament, or donor-derived glia. To immunostain donor neurofilaments, a monoclonal antibody to neurofilament 70,000 mol. wt. (rat adsorbed, NF70; Biodesign, Kennebunkport, ME) was used, and a monoclonal antibody against porcine cluster of differentiation antigen 44 (CD44; Diacrin, Inc., Charlestown, MA) was employed to identify donor glia. Free-floating sections were pretreated with 50% methanol and 0.3% hydrogen peroxide in PBS for 20 minutes, rinsed three times in PBS, and then pre-incubated for 1 hour in 10% normal blocking serum (NBS; normal goat serum for TH and NF70; normal horse serum for CD44) in PBS prior to overnight

incubation with primary antibody. TH antibody (Pel-Freez, Rogers, AK) was diluted 1:250 in PBS containing 1% normal goat serum, 1% bovine serum albumin, and 0.1% Triton-X 100, NF70 was diluted 1:1000 in PBS, and CD44 was diluted 1:2000 in PBS. Sections were then washed in PBS and incubated with goat anti-rabbit (TH; Vector Labs), goat anti-mouse (NF70; Sigma), or horse anti-mouse (CD44; Vector Labs) biotinylated secondary antibody diluted 1:200 (TH, CD44) or 1:1000 (NF 70) in 2% NBS in PBS for 90 minutes. After three PBS rinses, the sections were incubated with avidin-biotin complex (Vectastain ABC Kit ELITE, Vector Labs) for 90 min, rinsed once in PBS and twice in 0.05 M TBS, then developed with 0.04% hydrogen peroxide and 0.05% 3,3'-diaminobenzidine (Sigma) in TBS for 5-15 min.

In selected sections, donor-derived cells were identified via in situ hybridization with a digoxigenin-labelled PRE DNA probe which reacts specifically with porcine nuclei as described previously (Oettinger et al., 1995), with the following modifications for brain sections. Briefly, following TH immunohistochemical staining, sections were placed onto slides, allowed to dry, covered with pepsin, and incubated for 15 minutes at 50°C. Following two 5 minute rinses in 2x sodium chloride-sodium citrate (SSC), sections were post-fixed in 4% paraformaldehyde for 5 minutes at room temperature, rinsed two times in 2x SSC, and then dehydrated through an ethanol series. Slides were then dried and covered with a hybridization mixture containing a 234 bp PCR product digoxigenin-labelled PRE probe (25 ng/ml) diluted in Hybrisol VIII (1:700; Oncor, Inc., Gaithersburg, MD). The PRE probe was generated by PCR using primers flanking the repeat sequence and labelled with digoxigenin (Digoxigenin DNA Label Kit, Boehringer Mannheim, Indianapolis, IN). Sections

were covered with a glass coverslip and denatured at 100°C for 10 minutes. Following overnight hybridization at 37°C, slides were soaked in 2x SSC to remove the coverslips, and sections were rinsed for 10 minutes in a solution of 65% formamide in 2x SSC once at room temperature, once at 37°C, and once at 42°C. Sections were then rinsed twice in PBS and incubated at room temperature for 2 hours with an alkaline phosphatase-conjugated anti-digoxigenin antibody (1:200 in PBS; Boehringer-Mannheim, Indianapolis, IN). Following a 10 minute wash, sections were developed with 5-bromo-4-chloro-3-indolyl-phosphate/4-nitro-blue tetrazolium chloride (BCIP/NBT) substrate (Zymed, San Francisco, CA) and counterstained with nuclear fast red.

Morphometric and statistical analyses. Rotational data were analyzed by repeated measures analysis of variance (general linear model) with SAS 6.08 (SAS Institute, Cary, NC) followed by CONTRAST and Bonferonni adjustment for multiple comparisons. Non-linear regression was performed with DeltaGraph Professional 3.5 (Delta Point, Monterey, CA) using the Marquardt-Levenberg algorithm. TH+ neurons were counted in every sixth section of each graft and expressed as total number of TH neurons per graft using the Abercrombie formula (Abercrombie, 1946), and total graft volume was quantified by measuring CD44+ graft areas with the aid of a computer image analysis system (Image v. 1.52 for Macintosh) and integrating the cross-sectional areas across the graft. TH+ neuron survival and graft volume were compared between groups by unpaired t-test. A value of $p < 0.05$ was considered statistically significant.

Results

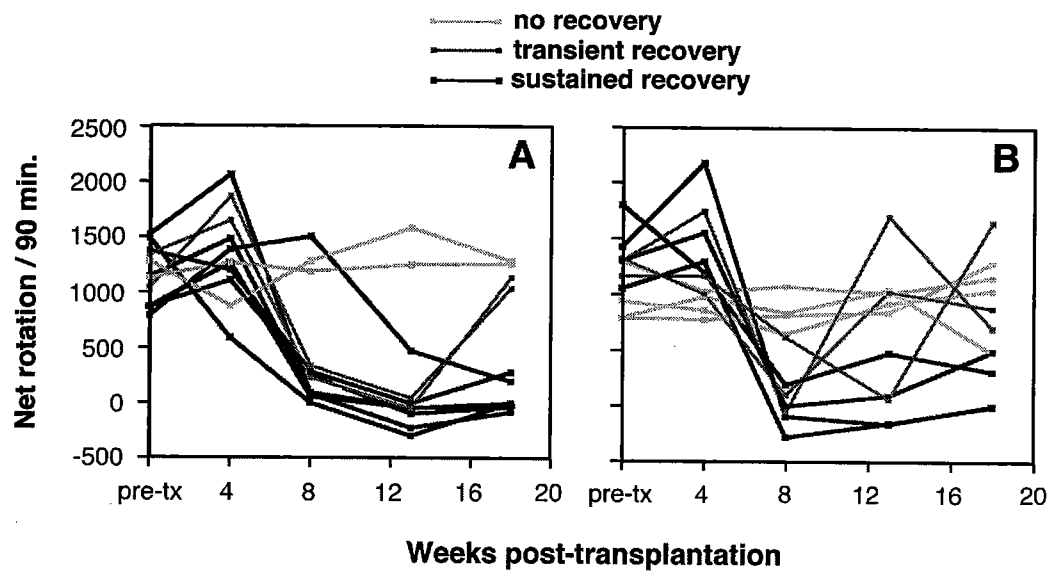
Effects of porcine ventral mesencephalic grafts on rotational asymmetry.

Amphetamine-induced rotational asymmetry was evaluated at 4-6 week intervals following transplantation of porcine fetal VM cells into the DA-depleted rat striatum. Individual net rotation scores for CyA and non-CyA treated rats are summarized in Figure 16. In the CyA treated group, 8 rats showed a greater than 50% decline in rotation at 8 weeks post-grafting relative to pre-transplant, and a ninth rat showed a reduction in rotation by 13 weeks. Two rats with behavioral evidence of functional grafts at 13 weeks returned to near pre-transplant baseline rotation levels by 19 weeks post-transplantation. Two additional CyA treated rats did not show any evidence of behavioral recovery during the course of the study.

A similar time-course of recovery was apparent in the non-CyA rats with 6 out of 11 rats showing a greater than 50% reduction in rotational asymmetry at 8 weeks post-transplantation. An additional rat showed functional graft effects at 13 weeks post-transplantation. However, at the end-point of the study, only 4 non-CyA rats maintained this reduction. Four of the rats in this group exhibited no evidence of functional recovery.

There was no significant difference in pre-transplantation net rotation scores between groups (unpaired t-test). There was a significant effect of time on rotational behavior across groups ($p = 0.0001$), yet there was not a significant group x time interaction. Within group analyses demonstrated that at 8 weeks following transplantation, both the CyA and non-CyA groups showed a

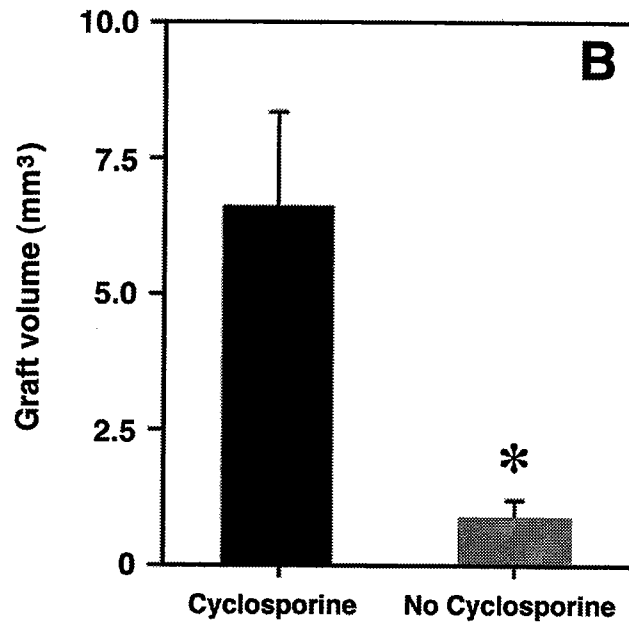
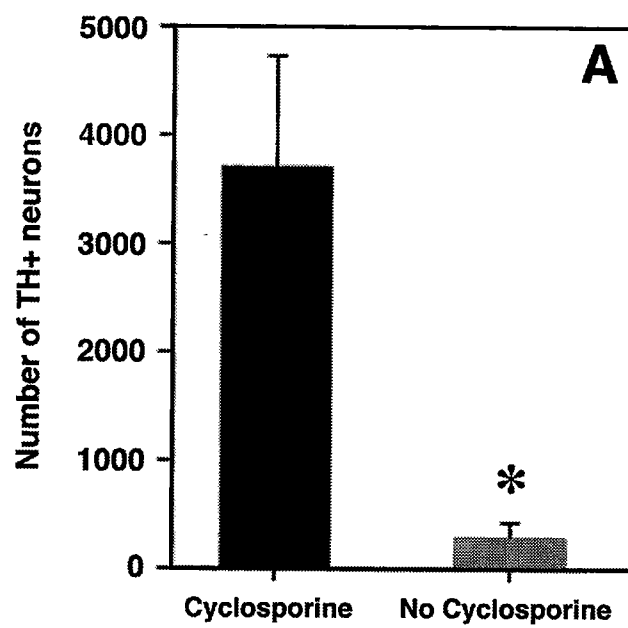
Figure 16. Amphetamine-induced (5 mg/kg, ip) net rotation asymmetry scores (turns ipsilateral to lesion minus turns contralateral to lesion during 90 minute period) plotted for individual rats pre-transplantation and at 4-6 week intervals post-transplantation. (A) CyA treated rats (n = 11). (B) Non-CyA treated rats (n = 11). In the CyA treated group, 7 rats showed sustained functional recovery (as defined by a greater than 50% reduction relative to pre-transplant value), 2 rats showed a transient reduction, and 2 rats showed no evidence of behavioral recovery. In the non-CyA treated rats, 4 rats showed sustained recovery, 3 rats showed a transient decline, and 4 rats showed no evidence of recovery. Dark grey lines represent rats showing sustained recovery, medium grey lines indicate transient recovery, and light grey lines indicate no evidence of recovery during the course of the study.



significant decrease in rotational asymmetry as compared to their respective pre-transplant values (CyA: pre = 1170.0 ± 76.9 , mean \pm SEM, wk 8 = 483.9 ± 167.8 , $p = 0.023$; No CyA: pre = 1205.4 ± 89.5 , wk 8 = 357.0 ± 138.6 , $p = 0.004$). For the CyA treated group, this attenuation of rotational deficit was maintained for the course of the study as indicated by the finding that the final rotation value at 19 weeks post-transplant (465 ± 178.2) did not significantly differ from that achieved at 8 weeks and remained significantly lower than the pre-transplant value ($p = 0.01$). In contrast, while the non-CyA group did show a significant decline in rotation at 8 weeks after grafting, this correction was not maintained at 19 weeks post-transplantation at which time the net rotation score (734.4 ± 160.1) was significantly greater than that observed at the 8 week recovery point ($p = 0.029$). Furthermore, at the endpoint of the study, the non-CyA group did not significantly differ from the pre-transplant baseline score.

Survival and size of porcine ventral mesencephalic grafts. Neural xenograft survival was assessed 19 weeks post-transplantation by TH immunohistochemistry and Nissl staining. Viable grafts, as defined by presence of surviving neurons, were found in 6 out of 11 CyA treated animals and 6 out of 11 in the non-CyA group. While there was no difference in rate of graft survival, the average number of surviving TH+ cell bodies in the grafts of the CyA treated rats (3690 ± 1023 , mean \pm SEM) was significantly greater than that in the non-CyA animals (257 ± 164 ; $p = 0.0078$, Figure 17A). A similar survival effect was evident upon measuring the graft volume of sections immunostained for the donor-specific glial marker CD44 (Figure 17B). Mean graft volume in the CyA

Figure 17. (A) Comparison of the number of surviving TH+ neurons in CyA treated and non-CyA treated porcine VM xenografts at 19 weeks post-transplantation. (B) Comparison of graft volume in CyA treated and non-CyA treated rats. Columns represent mean values. Bars represent SEM. * $p < 0.05$, unpaired t-test.



treated group ($6.59 \text{ mm}^3 \pm 1.73$) was significantly greater than that of the non-CyA rats ($0.826 \text{ mm}^3 \pm 0.33$, $p = 0.0084$).

Correlation between TH+ neuron survival and functional recovery.

Non-linear regression analysis of TH + neuron survival and extent of functional recovery, as measured by the change in net rotation, revealed a saturable relationship ($y = 104 x / [77 + x]$; $r^2 = 0.642$) indicating that survival of approximately 80 - 100 TH+ neurons is necessary to achieve a 50% reduction in net rotational asymmetry (Figure 18). This equation describes the close correspondence between the degree of functional recovery and the number of surviving neurons ($r = 0.80$, $p < 0.01$). At a survival of approximately 850 - 1000 TH+ neurons, behavioral recovery plateaued with additional neuronal survival providing no further effect on rotation. In each group there was one rat which showed evidence of behavioral recovery yet, which upon histological analysis, revealed no surviving grafts using available histologic techniques. These animals were not included in this correlation analysis.

Morphology and organization of fetal porcine ventral mesencephalic grafts.

In situ hybridization for PRE DNA demonstrated localization of the majority of porcine-derived nuclei within the confines of the VM graft. Donor-derived cells were uniformly distributed while the donor TH+ neurons formed clusters within the graft (Figure 19A). PRE DNA labelling revealed a distinct graft-host boundary (Figure 19B). Porcine cells were not found outside the graft in the host striatal grey matter yet were observed in host white matter tracts, particularly the internal capsule and corpus callosum. Since no TH+ or NF70+

Figure 18. Relationship between TH+ neuron survival and extent of functional recovery. Squares (n) represent individual rats from the CyA treated group, and circles (l) represent rats from the non-CyA treated group. Non-linear regression analysis revealed a saturable relationship fit to the equation $RR = RR_{max} * N / N_{50} + N$ where RR = rotational reduction; RR_{max} = maximum rotational reduction; N = number of TH+ neurons; N_{50} = number of TH+ neurons necessary to achieve a 50% reduction in rotation ($RR = 104 * N / [77 + N]$; $r^2 = 0.642$).

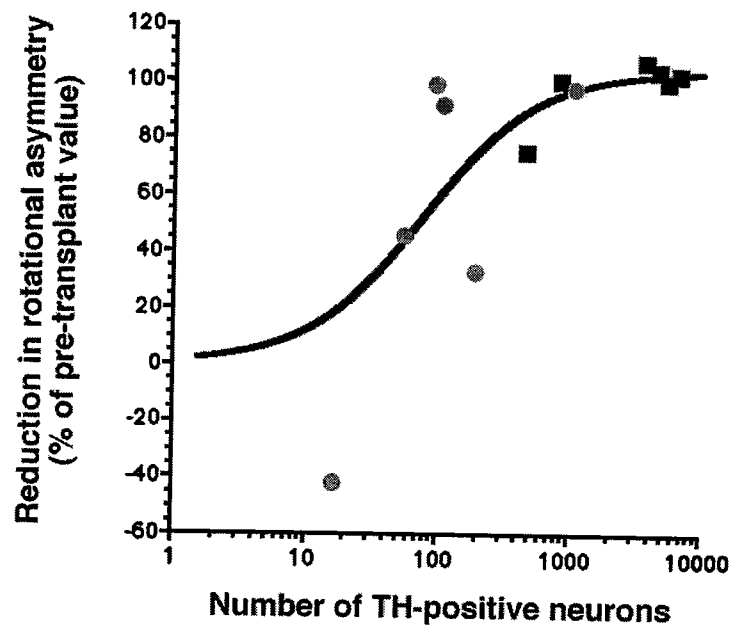
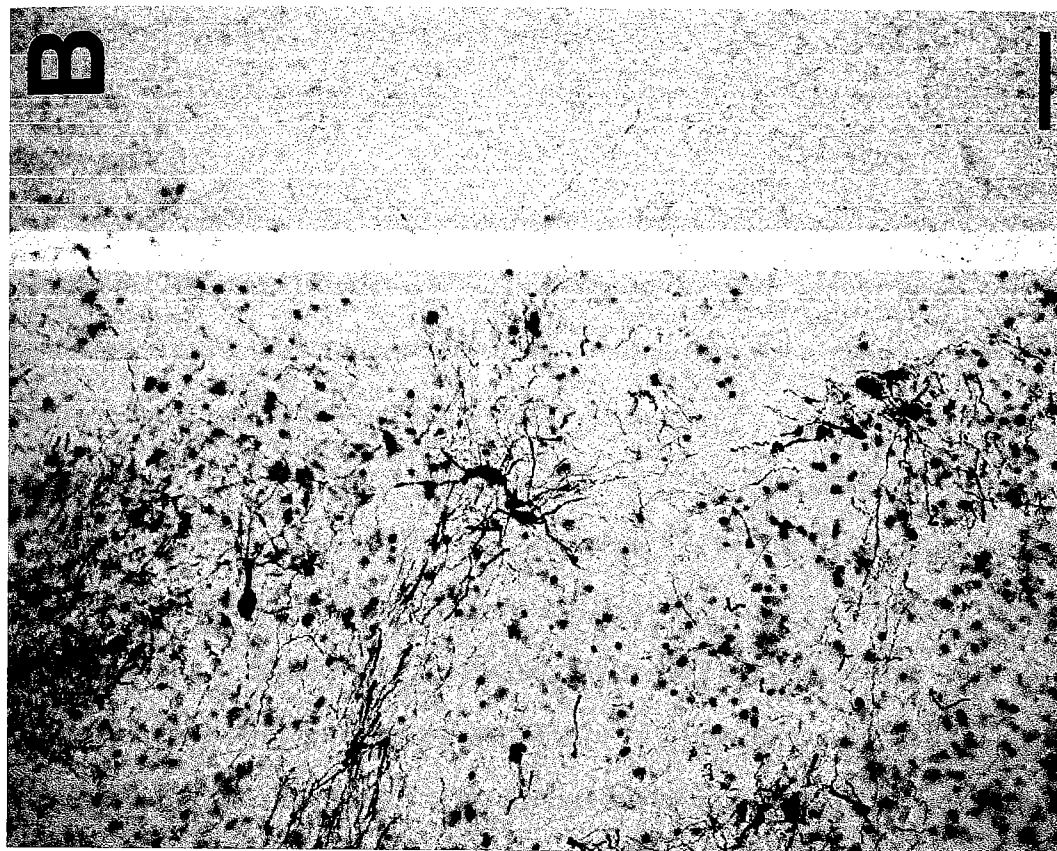
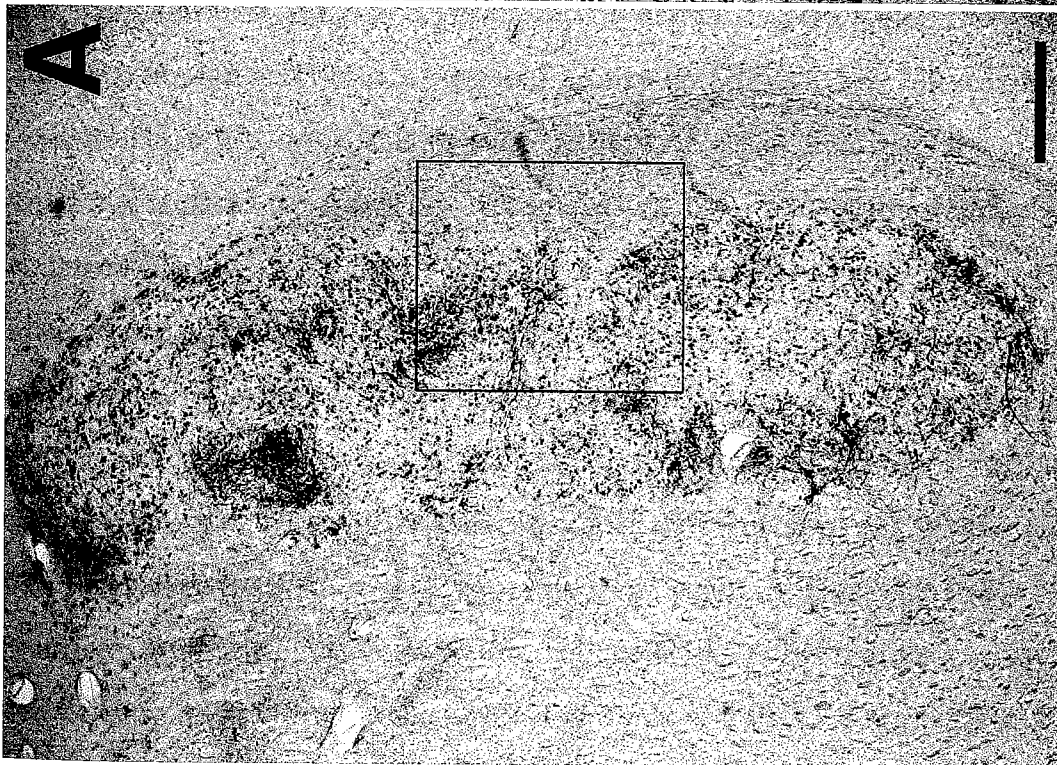


Figure 19. (A) Coronal section through intra-striatal VM graft in which donor-derived cells were identified by in situ hybridization using a probe which recognizes a pig-specific DNA repeat element. Section is double labelled with TH and counterstained with nuclear fast red. All blue dots are nuclei of pig-derived cells. The majority of the donor cells are located within the confines of the graft, while a fraction of the pig cells have migrated into the host white matter. (B) Higher magnification of the enclosed area shown in A. Note distinct graft-host boundary. Scale bar = 500 μm (A), 100 μm (B).



neuronal cell bodies were observed outside the graft, these cells most likely correspond to CD44+ glia which have migrated into the host white matter (Figure 19A, B).

In large grafts, the donor-derived TH+ fibers reinnervated the entire extent of the striatum (Figure 20B). Axons from the graft grew into the grey matter of the surrounding host striatum, avoiding the penetrating white matter tracts. This pattern of reinnervation was comparable to the normal striatal innervation by TH+ axons originating from the intact contralateral SN (Figure 20A), and the density of the TH+ fibers in the grafted striatum approached that observed in the intact striatum (Figure 20A). As shown in Figure 20C, TH+ neuron staining was absent in SN ipsilateral to the 6-OHDA lesion, indicating that the dense striatal innervation was entirely donor-derived. No TH+ axons were present in the striatum of rats without surviving grafts (data not shown).

The neuronal and glial organization of ventral mesencephalic xenografts in the CyA treated and non-CyA treated groups was assessed by Nissl staining as well as TH, NF70, and CD44 immunostaining. Nissl stained sections of CyA treated rats showed large, neuron-rich grafts which were well integrated with the host tissue (Figure 21A). The transplants contained large numbers of TH+ cell bodies with the characteristic morphology of SN DA neurons (Figs. 20B, 21B). Immunostaining of the porcine VM xenografts for TH revealed organotypic features of the SN, with large clusters of TH+ neurons at the graft perimeter and a dense network of TH+ neuronal fibers within the transplant and extending from the graft to innervate the surrounding host striatum (Figs. 20B, 21B). NF70 immunostaining further demonstrated that the TH-negative regions of the grafts were densely filled with porcine-derived neurons and fibers (Figure 21C). CD44

Figure 20. Dark-field photomicrographs from a representative grafted rat immunostained for TH. (A) Intact striatum. (B) Grafted striatum ipsilateral to previous 6-OHDA lesion. (C) VM of the same rat as shown in A and B. Note the absence of dopaminergic cell bodies in the SN and fibers in the striatum ipsilateral to the 6-OHDA lesion. Scale bars = 500 μ m.

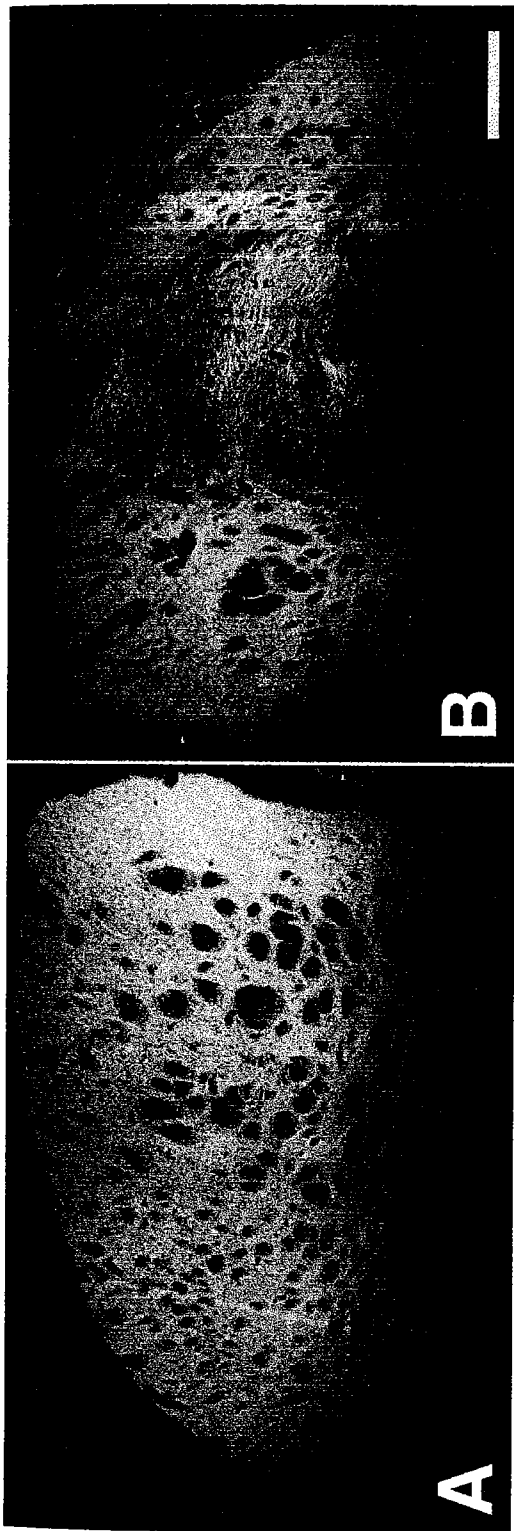
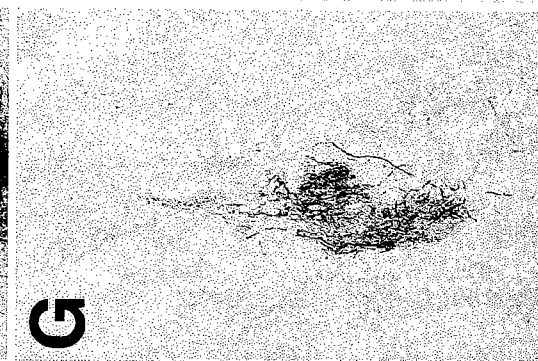
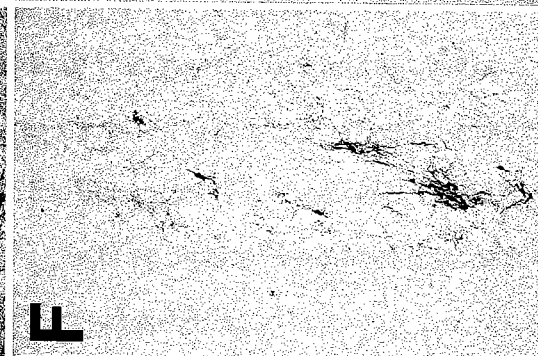
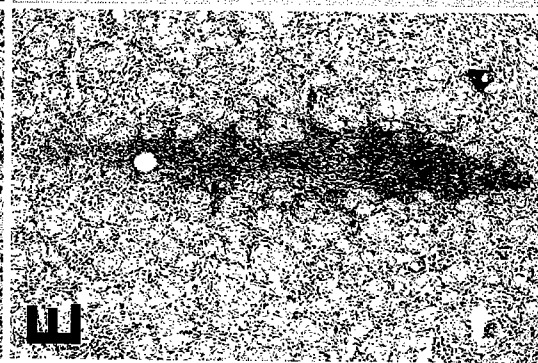
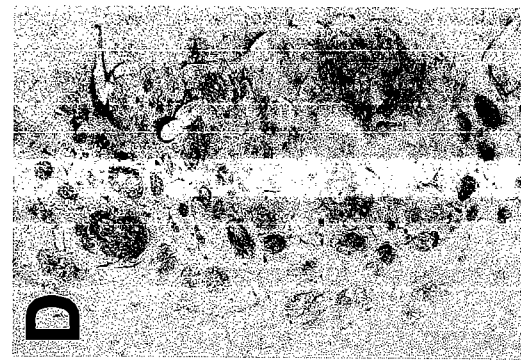
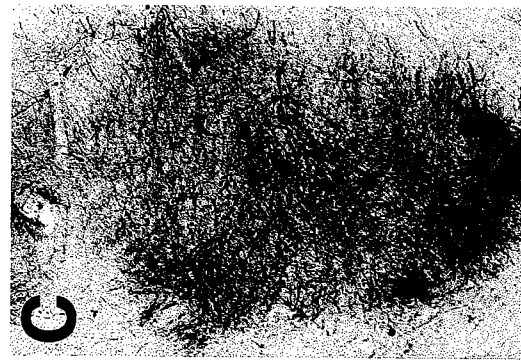
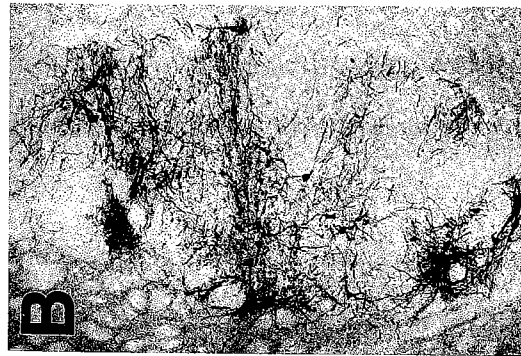
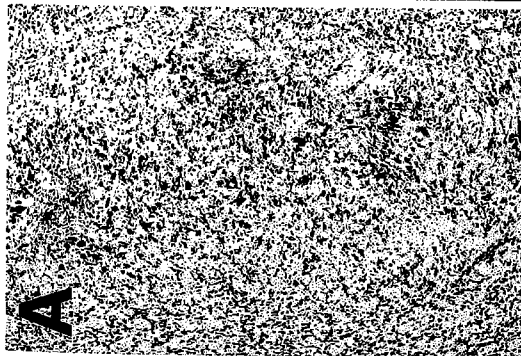


Figure 21. Adjacent coronal sections through a representative porcine VM xenograft of a CyA treated rat (A - D) and non-CyA treated rat (E - H) stained for Nissl (A, E), TH (B, F), NF70 (C, G), and CD44 (D, H). Refer to text for further description. Scale bar = 200 μ m.



immunostaining of donor-derived glia revealed a distinct glial component within the graft with the most dense labelling observed within the host white matter tracts penetrating the graft (Figure 21D). Nissl staining of the two CyA treated rats which showed loss of behavioral recovery revealed a small-cell infiltrate and a limited area of necrosis at the graft site. In addition, there was no evidence of neuronal graft survival in the two CyA treated rats which showed no behavioral compensation. A slight scar (approximately 50-100 μm in width) was present along the needle tract in these two animals.

In contrast to the CyA treated rats, grafts from non-CyA rats were characteristically small and condensed with few surviving neurons as demonstrated by the limited number of TH and NF70 immunostained cells (Figure 21E-G). Surviving grafts from this group contained varying degrees of small-cell infiltration. There was greater survival of the glial components of the graft relative to the neuronal components in this group. In contrast to the CyA treated rats, CD44+ immunostaining was usually concentrated in the graft core and fewer fibers were seen in the white matter tracts (Figure 21H). Of particular interest, no significant host tissue damage was apparent beyond the transplant site in rats that did not have surviving grafts at sacrifice.

Discussion

The present results demonstrate that ED 27 porcine VM neuroblasts transplanted into the DA-depleted rat striatum are able to reinnervate the host and produce functional recovery as measured by a reduction in amphetamine-

induced rotational asymmetry. These results are consistent with previous reports of porcine xenografts in rats (Huffaker et al., 1989). In the present study, the extent of behavioral recovery was found to be highly correlated with the number of surviving TH+ neurons. As demonstrated in other xenograft paradigms (Brundin et al., 1985c; Brundin et al., 1988b), immunosuppression with CyA increased survival of the xenografted TH+ neurons. Using donor-specific markers and hybridization to a porcine specific DNA repeat element, we show organotypic cellular organization of TH+ neurons and glial components within porcine VM grafts as well as extensive reinnervation of the DA-depleted host striatum.

Behavioral effects were apparent in both the CyA immunosuppressed and non-CyA rats at 8 weeks following transplantation suggesting the presence of functioning grafts at this time. A similar time course of recovery has been demonstrated previously following the transplantation of pig ED 21 VM cells into CyA treated rats (Huffaker et al., 1989). Previous studies evaluating behavioral recovery in rats following transplantation of mouse (Brundin et al., 1985c; Brundin et al., 1989), rat (Brundin et al., 1988a; Dunnett et al., 1983), or human (Brundin et al., 1986) dopaminergic neurons have shown the onset of recovery to be at approximately 3-4 weeks following grafting of mouse or rat tissue, and 15-19 weeks for human transplants. The intermediate time course of recovery associated with the grafting of porcine dopaminergic neurons probably reflects the developmental rate of the pig relative to rodents and humans (Dickerson and Dobbing, 1967; Sacher and Staffeldt, 1974; Snow and Tam, 1980).

While there was a significant decrease in amphetamine-induced rotation in both the CyA and non-CyA groups 8 weeks after grafting, this effect was not

sustained in the non-CyA rats. Indeed, at the endpoint of the study, the non-CyA group had returned to pre-transplant baseline rotation values. Conversely, at 19 weeks post-transplantation, rotation in the CyA group remained significantly lower than pre-transplant values and did not differ from scores seen at 8 weeks post-transplantation. The CyA group had significantly greater TH+ neuron survival and greater graft volume as compared to the non-CyA group. These results demonstrate that under CyA treatment, porcine VM grafts are able to survive long-term in the adult host brain and produce sustained functional recovery on amphetamine-induced rotation.

There was a high degree of correlation between the number of surviving grafted TH+ neurons and the extent of behavioral recovery. Regression analysis revealed a threshold number of approximately 80-100 neurons necessary to obtain at least a 50% reduction in amphetamine-induced rotation. It has been determined that only 3% restoration of striatal DA is necessary for recovery on amphetamine-induced rotation (Schmidt et al., 1983; Schmidt et al., 1982). Similar analyses of allografts in the 6-OHDA rat model of PD have demonstrated a minimum of approximately 100-200 surviving rat DA neurons in order for a 50% reduction in rotational asymmetry (Brundin et al., 1985b). The present recovery at a neuron number of 80-100 may reflect a larger degree of striatal reinnervation per porcine neuron or a greater DA output per neuron. Similar to previous studies, a plateau in rotational recovery was found beyond which further compensation did not occur (Björklund et al., 1980a; Brundin et al., 1988a; Huffaker et al., 1989) which may be attributable to the autoregulatory capacity of the transplanted dopaminergic neurons or maximal behavioral response (Strecker et al., 1987).

The specific histological analysis of surviving grafts in the CyA treated group showed neuron-rich grafts with TH+ cell bodies located primarily at the graft-host interface. TH+ axons and dendrites were present within the graft, and axons extended from the graft to densely reinnervate the grey matter of the entire grafted striatum. The complete loss of TH+ neurons in the SN associated with the 6-OHDA lesion (Figure 20C) indicates that these TH+ fibers were entirely donor-derived. NF70 was used to identify both the dopaminergic and non-dopaminergic grafted neurons and revealed NF70+ neurons within the TH-negative regions of the graft. Similar to the dopaminergic reinnervation, NF70+ axons extended into the host striatum as well as into more distant targets (Isacson et al., 1995). Neuronal cell bodies were not found outside the grafted area. CD44 immunostaining revealed donor glia distributed predominantly in striatal white matter tracts within and around the graft. In addition, glial fibers and cell bodies were apparent in distant white matter tracts. Using a pig-specific DNA marker, the relative extent of donor-derived cells was shown to be dense throughout the graft with some migration of cells into the surrounding striatal white matter as well as into the corpus callosum of the contralateral hemisphere. We have previously shown the growth of glial fibers and migration of CD44+ cells in these host white matter tracts (Isacson et al., 1995), and the present porcine specific DNA-labelling confirms the migratory capacity of donor glia within host white matter (Zhou and Lund, 1993).

In contrast, the majority of grafts in the non-CyA treated rats were small and contained a sparse distribution of neurons. Interestingly, the glial component of the graft was relatively preserved whereas the neuronal component was lost. Cellular infiltrates and perivascular cuffing were often

present. It is unclear if these non-CyA treated grafts would persist over time, yet the contrast in graft appearance between the two groups indicates the importance of CyA treatment on graft survival and the associated functional recovery. Similar to previous reports, brains in which grafts presumably died early contained only minor scars along the injection tract whereas a few grafts showed a small necrotic area with cellular infiltration (Brundin et al., 1988b). Of significance to clinical transplantation, the area of damage was confined to the graft site and did not extend to the surrounding striatum.

Behavioral evidence of functional grafts as well as long-term neuronal survival were present in some of the non-CyA treated rats in the present study, and a moderate degree of prolonged neural xenograft survival in non-immunosuppressed rats has been reported previously (Björklund et al., 1982; Brundin et al., 1985c; Daniloff et al., 1985). However, the fact that the immunological privilege of the brain is not absolute (Pakzaban and Isacson, 1994; Widner and Brundin, 1988) necessitates immunosuppression of the host for xenograft survival. The administration of CyA has been demonstrated to enhance neural xenograft survival (Brundin et al., 1985c; Brundin et al., 1988b), presumably due to the inhibition of IL-2 production and subsequent T cell activation (Bierer et al., 1993). In addition, recent data show that the immunosuppressants CyA and FK506 decrease NMDA-mediated glutamate toxicity in cortical cultures (Dawson et al., 1993). Yet, neither CyA nor FK506 have been shown to have an effect on neuronal survival in the absence of a toxic insult *in vitro* (Dawson et al., 1993). While it is unclear to what degree these results can accurately be extrapolated to grafted neurons, these trophic effects

may be synergistic with the well known immunosuppressive effects of CyA on graft survival.

Alternatives and adjuncts to CyA immunosuppression are under investigation. While steroids and azathioprine are used in some clinical neurotransplantation protocols (Lindvall et al., 1994; Widner et al., 1992), their precise therapeutic benefit for neural grafting remains unclear. A single dose of the anti-inflammatory steroid methylprednisolone (5 mg/kg) was administered preoperatively in the present study. A recent study in rodents has demonstrated that 30 mg/kg/d of methylprednisolone enhances graft survival whereas a lower dose of 15 mg/kg/d results in the majority of the grafts being rejected (Duan et al., 1996). The use of a triple drug regimen comprised of CyA, prednisolone, and azathioprine has been reported to increase the survival rate of xenografted hippocampal tissue blocks in rats (Pedersen et al., 1995). Alternatively, it has been reported that corticosteroid treatment may reduce graft survival (Patino et al., 1992). Thus, further studies are necessary to define clearly the effects of steroid immunosuppression in CNS grafting. As an alternative to drug therapies, treatment with antibodies to the interleukin II receptor has been shown to protect against immune rejection in human-to-rat VM grafting (Honey et al., 1990). In addition, prior masking of donor MHC I on transplanted cells enhances the frequency of neural xenograft survival relative to no immunosuppression in a rat model of Huntington's disease (Pakzaban et al., 1995). Yet, such masking is not equivalent to CyA as graft size was significantly reduced in the masked group relative to the CyA treated group. We are currently evaluating the effects of MHC I masking of porcine VM cells as well as other immunomodulatory techniques on graft survival in the rat Parkinson model.

Current human fetal transplantation paradigms are restricted by several practical issues, the foremost of which is limited tissue supply. In agreement with previous studies (Freeman et al., 1988; Huffaker et al., 1989), the present results demonstrate that transplanted porcine fetal dopaminergic neurons are able to reinnervate the brain of immunosuppressed adult rats and mediate functional recovery. Thus, large quantities of age-appropriate DA neurons can be isolated and transplanted to the host brain where synaptic circuitry can be established resulting in regulated DA release. This long-term graft survival and extensive striatal reinnervation demonstrate the potential utility of transplanting fetal porcine DA neurons for the treatment of PD.

ACKNOWLEDGEMENT

All studies presented in this chapter were conducted by the candidate with the exception of the *in situ* hybridization procedure which was performed in collaboration with Dr. Jonathan Dinsmore. The material presented in this chapter is in press:

Galpern, W. R., Burns, L. H., Deacon, T. W., Dinsmore, J. and Isacson, O. (1996) Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: Functional recovery and graft morphology. *Experimental Neurology*, in press.

CHAPTER VI

GENERAL DISCUSSION

Therapeutic approaches to the treatment of Parkinson's disease

Limitations of current therapies for Parkinson's disease. Current therapies for PD are based primarily on the pharmacologic replacement of DA by the administration of L-dopa. Recently, surgical interventions including pallidotomy and fetal cell transplantation have become more widespread. However, each of these approaches has limitations as L-dopa therapy is associated with debilitating motor abnormalities and decreased efficacy whereas the long-term clinical outcome from pallidotomy is largely unknown, and transplantation of fetal neurons is associated with practical issues regarding attainment of sufficient tissue.

Since PD is characterized primarily by the loss of a single neurotransmitter system, pharmacological replacement of DA is of significant clinical benefit. However, while the development of L-dopa therapy is considered to be revolutionary in the treatment of PD, disease progression persists, and long-term treatment is associated with decreased efficacy as well as several adverse effects. Motor fluctuations associated with long-term L-dopa treatment are thought to be attributable in part to decreased DA buffering capacity in the brain due to loss of nerve terminals (Chase et al., 1993). Thus, mere DA replacement is not sufficient to achieve optimal therapeutic benefit as it is thought that fluctuating brain DA

levels contribute to the observed motor abnormalities. The benefits of L-dopa often decline after approximately 3 years of treatment (Marsden and Parkes, 1977; Yahr, 1977). Furthermore, evidence suggests that L-dopa itself may contribute to disease progression by producing reactive oxygen species which exacerbate ongoing neurodegenerative processes (Spina and Cohen, 1989). L-dopa and other dopaminergic agonists clearly improve the quality of life for parkinsonian patients initially. However, the disease cannot be adequately managed long-term using available pharmacologic agents, and present therapies do not halt disease progression.

Surgical approaches for the treatment of PD are aimed either at replacing striatal DA via transplantation of fetal dopaminergic neurons or interrupting the circuitry of the basal ganglia by lesioning brain structures. While initial outcomes from pallidotomies are encouraging, it is unclear if the observed therapeutic benefit will be sustained. Transplantation of human fetal VM into the DA depleted striatum of parkinsonian patients has yielded very promising results thus far. As discussed in Chapter 5 and below, the major constraint encountered in transplantation is obtaining sufficient tissue for grafting. In addition, several factors including graft site and placement as well as optimal number of cells and immunosuppression protocols have not yet been conclusively determined. Furthermore, as only approximately 10% of grafted cells survive following implantation, and only 5-10% of these cells are dopaminergic (Brundin et al., 1985b), development of strategies to enhance survival of transplanted neurons is of great interest.

Alternative approaches to the treatment of Parkinson's disease. The optimal approach to the treatment of neurodegenerative disorders would halt disease progression by preventing further neuronal death. As presented in Chapters 2 and 3, NTFs have been shown to protect against neuronal loss in animal models of nigral degeneration. Yet, several issues remain to be addressed before NTF delivery to the adult brain may be realized as a clinical alternative. Furthermore, the mechanisms underlying NTF protection are largely unknown. As shown in Chapter 4, NTFs likely act in part by decreasing the formation of reactive oxygen species. Until neuroprotective therapies are developed, DA replacement via transplantation of fetal mesencephalic neurons offers significant hope for PD patients. Since current procedures are limited by practical as well as ethical issues, we have investigated the use of fetal porcine VM as an alternative cell source for transplantation in PD. As demonstrated in Chapter 5, xenografts are able to survive in the immunosuppressed rat brain and mediate functional recovery. However, as discussed below, issues of immunosuppression and approaches to enhancing neuronal survival deserve further investigation.

Experimental approaches to neuroprotection of dopaminergic neurons

Neuroprotection in a rat model of Parkinson's disease. Using an MPP+ model of SN degeneration, we have shown that BDNF delivered by genetically engineered fibroblasts is able to protect against dopaminergic neuronal degeneration associated with inhibition of mitochondrial complex I (Chapter 2).

Furthermore, the present results indicate that BDNF is able to increase DA levels in the SN in this same lesion paradigm (Chapter 3). Demonstration of neuroprotection by BDNF suggests that this factor may be able to retard or halt ongoing neurodegenerative processes. Moreover, the augmentation of DA associated with BDNF delivery suggests that this NTF may also be able to enhance the function of surviving dopaminergic neurons and increase DA levels in remaining cells.

The MPP+ model of SN degeneration employed in these studies is useful for the understanding of the potential therapeutic role of NTFs. MPTP administration causes a parkinsonian syndrome in humans as a result of inhibition of complex I of the mitochondrial electron transport chain by its active metabolite MPP+ (Langston et al., 1983). Evidence for impaired complex I activity has been found in PD patients (e.g., Parker et al., 1989; Schapira et al., 1990; Schapira et al., 1989; Shoffner et al., 1991) suggesting that the neuronal death induced by MPP+ may accurately model the neurodegenerative processes underlying PD. Demonstration of effective neuroprotection in the MPP+ paradigm may therefore have direct relevance to the pathogenesis and treatment of PD. It has been suggested that neurodegenerative diseases may be associated with trophic factor deficiency (Appel, 1981; Hefti, 1983). While no mutation in the BDNF gene has been identified in cases of autosomal parkinsonism (Gasser et al., 1994), it remains possible that levels are deficient or that receptor interactions may be altered in the parkinsonian brain. Similar to the trophic factor dependence observed for developing neurons, adult neurons also may be dependent on trophic support, and lack of such a factor may result in impaired cellular function culminating in neuronal death.

In the studies presented in Chapters 2 and 3, the observed neuroprotective effects are mediated by a BDNF dose which is substantially lower than the dose employed in many infusion studies (e.g., Altar et al., 1992; Martin-Iverson et al., 1994; Sauer et al., 1993). This observation suggests that there may be a dose-response curve for BDNF efficacy. Furthermore, it is possible that the delivery via cells is more effective due to combined effects with baseline levels of other NTFs secreted by the fibroblasts. Unfortunately, the MPP+ model precludes behavioral testing of the effects of BDNF as a large striatal lesion is produced following infusion of this toxin. It would be of interest to determine not only the behavioral effects of BDNF but also the striatal neurochemical parameters associated with cell mediated BDNF delivery. It has recently been reported that GDNF delivered intraventricularly to primates lesioned unilaterally with MPTP is associated with remarkable behavioral improvement (Gash et al., 1996). Interestingly, while these behavioral signs were accompanied by increased DA levels in the SN, VTA and pallidum, DA levels in the caudate and putamen were not restored. Further characterization of the neurochemical and behavioral properties of trophic factors as well as the associated cellular and receptor alterations are necessary to fully address the clinical implications of the observed effects.

In addition to the findings presented in Chapters 2 and 3, studies have demonstrated that infusions of BDNF protect against the cell death associated with axotomy (Hagg, 1994), and BDNF-secreting fibroblasts have been shown to protect against neuronal degeneration associated with striatal injection of 6-OHDA (Levivier et al., 1995). While the induction of cell death differs in these paradigms, these findings confirm that BDNF is neuroprotective *in vivo*. The

mechanism of neuroprotection by BDNF has not yet been elucidated. As discussed previously, neuronal death may occur as a result of various cellular insults, culminating in the activation of cellular proteases and the production of free radicals. As discussed below, it is thus possible that BDNF as well as other NTFs protect against cellular damage by upregulating the activity of protective mechanisms. Indeed, BDNF has been shown to increase levels of the antioxidant glutathione reductase *in vitro* (Spina et al., 1992).

In addition to BDNF, several other NTFs have been shown to be trophic for dopaminergic neurons. As many of these factors are members of different gene families and act via different receptors, it is likely that they may induce different cellular responses. Thus, combined treatment with more than a single factor may produce additive or synergistic effects. Indeed, in the wobbler mouse model of motor neuron disease, the combined administration of BDNF and CNTF provided significantly greater protection against neuronal loss than either factor alone (Mitsumoto et al., 1994).

PD is well-suited for neuroprotective therapy as primarily one cell population is affected and the clinical manifestations are not apparent until 80-85% of SN dopaminergic cells have degenerated and striatal DA levels are depleted by 80% (Marsden, 1982a). PET scan studies have demonstrated that nigral cell loss can be detected during the preclinical stages of PD (Brooks, 1991; Burn et al., 1992). Thus, identification of patients during the subclinical stage in combination with neuroprotective therapies could theoretically enable the prevention of disease progression. At present, however, PET scans are not widely available. Moreover, further characterization of NTFs is necessary before clinical applications are feasible. To date, no clear preventative benefit has been

reported in clinical trials of NTFs, and high doses have been associated with significant toxicity (Miller et al., 1996). As discussed below, several factors must be evaluated in order to consider the therapeutic applications of NTFs.

Mechanism of neuroprotection in a rat model of mitochondrial impairment. The mechanism underlying the BDNF-mediated neuroprotection in the MPP+ model is not yet known. In order to investigate the mechanism of NTF-mediated neuroprotection, we studied the effects of NGF in a 3-NP model of striatal degeneration. 3-NP, like MPP+, is a mitochondrial toxin. By inhibiting complex II of the electron transport chain, 3-NP produces striatal cell loss. The use of this model for the mechanistic evaluation of NTF-mediated neuroprotection is advantageous as more tissue (striatum versus SN) is available for analysis. While the MPP+ and 3-NP models differ in their neuropathology, both act via mitochondrial blockade. The use of the paradigm presented in Chapter 4 has enabled us to evaluate NTF protective mechanisms *in vivo*. However, the differences in the MPP+ and 3-NP models as well as the trophic factors evaluated clearly distinguish the paradigms. Subsequent studies will be focused on similar evaluation in the MPP+ model, likely using tissue pooled from treatment groups.

As demonstrated in Chapter 4, NGF is able to attenuate the production of 3-nitrotyrosine *in vivo*. 3-nitrotyrosine is the product of peroxynitrite-mediated tyrosine nitration and thus serves as an indirect measure of peroxynitrite activity. The observed reduction in 3-NP induced generation of 3-nitrotyrosine in the NGF treated group suggests that NGF acts in part by reducing peroxynitrite formation. Thus, NGF may increase SOD or decrease NO levels, thereby

decreasing peroxynitrite. These findings not only demonstrate one mechanism by which NGF is able to protect against 3-NP toxicity, but also suggest that therapeutic agents targeting NOS inhibition or enhancing SOD levels may be able to protect against oxidative stress in neurodegeneration.

Similar to 3-NP, MPTP has also been reported to increase the generation of 3-nitrotyrosine *in vivo* (Schulz et al., 1995b). Furthermore, treatment with the neuronal nitric oxide synthase inhibitor 7-nitroindazole was shown to protect against the MPTP-induced reduction in DA as well as the increase in 3-nitrotyrosine suggesting that NO and peroxynitrite are important mediators of MPTP toxicity. Thus, BDNF protection against MPP⁺ toxicity may also involve decreased formation of peroxynitrite.

Considerations for clinical use of neurotrophic factors. The trophic support and neuroprotection afforded by NTFs indicate the possible therapeutic applicability of these factors to neurodegenerative disorders (Hefti, 1994; Jelsma and Aguayo, 1994; Lindsay et al., 1993). However, the clinical application of NTFs as a therapeutic modality for neurodegenerative disorders requires an effective means of delivery. As NTFs cannot cross the BBB, alternate strategies for delivery to the CNS must be considered.

Various approaches to circumventing the BBB have been investigated. Direct intraparenchymal or intraventricular infusions have been employed in numerous experimental paradigms. While this method of delivery allows for dosage control and site-specificity, there is often parenchymal damage at the cannula placement site. Also, the delivery efficiency of factors may be limited by diffusion properties of the factor within brain parenchyma (Morse et al., 1993).

Additionally, intraventricular administration of the TrkB ligand BDNF is ineffective due to binding by truncated TrkB receptors which are present in the ependymal lining of the ventricles (Kordower et al., 1994b). Similar difficulties may be expected for NT-4/5 as this factor also binds to the TrkB receptor. Continuous delivery could be achieved by a pump reservoir connected to a cannula. However, growth factor stability in the reservoir may be of concern. Moreover, approaches involving intracerebral cannulae introduce a high risk for infection. An additional approach to delivery would be to modify the NTF in order to enable BBB passage. It has been shown that NGF-transferrin receptor antibody conjugates are able to cross the BBB and protect neurons from excitotoxic lesions suggesting that such an approach may provide a non-invasive route of delivery to the brain (Friden et al., 1993; Kordower et al., 1994a).

As an alternative to direct infusion, methods for cell-mediated delivery of therapeutic proteins have been developed including *ex vivo* as well as *in vivo* gene transfer. *Ex vivo* gene transfer involves the genetic engineering of cells to express a transgene prior to transplantation (see Gage et al., 1987 for review). Intracerebral grafting of primary or immortalized fibroblasts genetically engineered to release trophic factors allows for site specific biological delivery which has been shown to prevent cell loss in a variety of *in vivo* lesion paradigms (e.g., Kawaja et al., 1992; Rosenberg et al., 1988; Schumacher et al., 1991). In contrast to the use of immortalized fibroblast lines, the use of primary fibroblasts decreases the likelihood of tumor formation following implantation and would allow for harvesting cells from the affected individual, thereby eliminating the possibility of immune rejection. Alternatively, cell division of immortalized fibroblasts could be arrested prior to implantation or cells could be encapsulated

in a semipermeable membrane (Winn et al., 1994). Difficulties with achieving stable, long-term gene expression by genetically modified cells remain an obstacle to this approach as gene expression is down-regulated post-implantation (Palmer et al., 1991). Improved vector constructs with alternate promoters may resolve this issue. Moreover, the use of inducible promoters (Gossen and Bujard, 1992) may allow for regulated factor delivery by the genetically modified cells. Virus vectors have also been used to infect human neural progenitors prior to transplantation (Martinez-Serrano et al., 1995; Sabaté et al., 1995) suggesting the potential utility of *ex vivo* genetic engineering of progenitor cells.

Several groups have attempted to infect cells *in vivo* via herpes simplex virus (Breakefield and DeLuca, 1991), adenovirus (Horellou et al., 1994; Le Gal La Salle et al., 1993), or adeno-associated virus (Kaplitt et al., 1994) vectors. While these approaches of genetic modification of cells *in vivo* holds promise for future therapeutic interventions, current methodologies are constrained by limited gene expression as well as by the cytopathogenicity and the low rate of infectivity associated with virus vectors.

Optimal methods of delivery may vary for NTFs due to receptor distribution and factor properties. Moreover, for each growth factor, effective dose ranges need to be determined as the dose-response relationship for many factors indicates higher doses may be less effective. Indeed, such a dose-response effect has been reported *in vitro* for the trophic effects of NT-3 and NT-4/5 on DA neurons (Hyman et al., 1994), and studies of axotomized SN neurons as well as axotomized motor neuron rescue demonstrate a submaximal response with higher doses of BDNF (Hagg, 1994; Vejsada et al., 1994). As suggested by Vejsada, et al. (1994), high levels of ligand may result in decreased affinity or

down-regulation of the high affinity TrkB receptor or an upregulation of the inactive truncated TrkB receptor, thereby accounting for the decreased efficacy of higher doses of BDNF.

Experimental approaches to neurotransplantation of dopaminergic neurons

Transplantation of fetal porcine dopaminergic neurons. The primary limitations to neuronal grafting are the limited supply of tissue and the low yield of surviving dopaminergic neurons. In order to investigate the use of alternate cell sources for transplantation in PD, we have investigated the survival and function of fetal porcine VM neuroblasts grafted in the 6-OHDA rat model of PD. As demonstrated in Chapter 5, following transplantation of porcine dopaminergic neurons to the denervated striatum, CyA treated rats exhibited sustained functional recovery, as measured by a reduction in amphetamine induced rotation, whereas non-CyA treated rats showed only a transient decline in rotation. The degree of rotational recovery was found to correlate with the number of surviving TH neurons in the grafts, and it was determined that a survival of approximately 80-100 TH+ neurons was necessary to achieve a 50% reduction in net rotational asymmetry. Remarkably, near complete graft-derived striatal reinnervation was observed in the CyA treated rats. These findings suggest that fetal porcine neuroblasts may provide an alternate source of dopaminergic neurons for transplantation in PD.

Properties of fetal porcine grafts. Histological analysis of the porcine grafts revealed extensive reinnervation of the striatum ipsilateral to the lesion. Similar to human-to-rat grafting studies (Wictorin et al., 1992), additional histological evaluation of the porcine xenografts has revealed target-specific long distance axonal growth by the transplanted neuroblasts (Isacson et al., 1995). Thus, despite species differences, the grafted cells are able to reconstruct neuronal circuitry and mediate functional recovery in the immunosuppressed adult brain.

While grafted dopaminergic neurons are able to compensate for many lesion-induced deficits, this recovery is incomplete as, for example, complex limb use measured by paw reaching ability as well as the adipsia and aphagia induced by bilateral lesions are not improved by rat-to-rat grafting (Björklund et al., 1980a; Dunnett et al., 1981b; Dunnett et al., 1987). Several factors have been suggested to underlie this lack of recovery, including insufficient striatal reinnervation, incomplete integration with the host circuitry, as well as failure to reinnervate non-striatal target areas (see Björklund, 1992 for review). It has, however, been shown the more extensive reinnervation and integration produced by micrografting of fetal neurons results in recovery on the paw reaching task (Nikkhah et al., 1994; Nikkhah et al., 1993a). As the porcine grafts in the present study demonstrated such remarkable fiber outgrowth and near complete striatal innervation, it would be of interest to determine if these grafts are also able to compensate for lesion induced paw reaching deficits.

Neurochemical analyses of fetal rat grafts have demonstrated that only 3% of normal DA levels are necessary for rotational recovery. Striatal DA levels are restored by approximately 13% following macrografting and by 30% using

micrografting techniques (Nikkhah et al., 1994). Analysis of DA levels in the porcine grafts would be useful to evaluate the comparative efficacy of fetal porcine dopaminergic neurons relative to the micrografting or macrografting of fetal rat tissue. The finding that only 80-100 porcine neurons are necessary to achieve rotational recovery whereas 100-200 rats neurons are needed (Brundin et al., 1985b) suggests that the porcine cells may provide more DA.

Xenotransplantation and immunoprotective strategies. While the porcine xenografts were able to survive and attenuate rotational deficits in 6-OHDA lesioned rats, the rate of graft survival in the CyA treated rats was only 55%. This observation suggests that in addition to cell-mediated responses, which are inhibited by CyA, other immunological factors may be involved. It has been suggested that humoral immunity may be important in xenograft rejection by activating complement and promoting antibody-dependent cellular cytotoxicity. However, determination of the role of antibodies in neural xenograft rejection awaits detailed characterization. The observed 55% survival rate is in agreement with the finding that the immunoprotection afforded by CyA is often incomplete (Pakzaban and Isacson, 1994) and indicates the importance of developing alternate immunosuppression strategies.

The 6-OHDA model allows for indirect monitoring of graft survival via rotational testing during graft growth and maturation. However, the onset of behavioral recovery following transplantation of porcine neurons does not occur until approximately 9 weeks after grafting. Thus, if a graft is rejected late after transplantation, the loss of neuronal function may be detected. However, there is no indicator of graft health prior to the onset of behavioral recovery, and

characterization of immunological reactions at the precise time of rejection may not be possible if the graft is rejected early.

As mentioned previously, triple drug therapy (Pedersen et al., 1995) as well as methylprednisolone alone (Duan et al., 1996) have been shown to effectively prevent mouse to rat xenograft rejection. It is of interest to determine the optimal immunosuppression regimen for xenografting to the CNS, and further evaluation is warranted. In addition to the conventional pharmacotherapies, novel immunosuppressive strategies directed at various points in the immune response are under investigation. As discussed previously, the cytokine IL-2, which is secreted by T helper cells, is important in the regulation of immune responses. By administering an antibody to the IL-2 receptor, IL-2 is prevented from binding to its receptor and mediating rejection. Ten day administration of IL-2 receptor antibody to rats receiving intraventricular solid allografts has been demonstrated to significantly increase graft survival and attenuate the cellular infiltrate and MHC upregulation observed in control allografted rats (Wood et al., 1992). Moreover, such treatment has been shown to effectively prevent xenograft rejection of human to rat transplants (Honey et al., 1990). Allograft rejection has also been shown to be prevented by immunoselecting and transplanting neuronal precursors which do not express MHC antigens (Bartlett et al., 1990). Masking of donor MHC I on cells prior to grafting has also been shown to be immunoprotective (Pakzaban et al., 1995). Yet, the graft volume in the masked group was found to be smaller than that in the CyA treated group. Experiments depleting either CD4 (T helper) or CD8 (cytotoxic) T cells in allografts or concordant neural xenografts have demonstrated that CD4 cells are critical mediators of graft rejection and that

xenograft rejection cannot be mediated by CD8 cells alone (Nicholas et al., 1990; Wood et al., 1996). Grafts in CD4 T cell depleted hosts were found to survive significantly longer relative to untreated or CD8 depleted hosts. The combined depletion of CD4 and CD8 cells resulted in greater graft survival, demonstrating that CD8 cells are indeed involved in the rejection process. Similar, yet less dramatic, immunoprotection by CD4 cell depletion was also observed in discordant neural xenografts (Wood et al., 1996).

Such manipulations of the immune system and of transplanted tissue, either alone or in conjunction with currently used immunosuppressive drugs, may provide enhanced immunoprotection. Further evaluation and characterization of the xenograft rejection process by analysis of cellular infiltrates and cytokine profiles within the graft will likely facilitate the development of additional strategies to prevent xenograft rejection.

Strategies to increase neuronal survival. As addressed in Chapter 5, a major constraint to fetal transplantation is the limited supply of tissue. Long-term storage of tissue prior to transplantation is associated with reduced neuronal survival and at present is thus not a suitable technique. In addition to using cells from a xenogeneic source, a further approach to circumventing the current grafting limitations is to enhance the yield of surviving dopaminergic neurons presently achieved by standard cell suspension grafting. Approximately 10% of the neurons in the VM are dopaminergic. However, it is estimated that only 10% of the grafted neurons survive, thereby requiring up to 10-15 fetal VMs to adequately reinnervate the parkinsonian striatum (Björklund, 1993).

As described, NTFs are able to promote the survival of dopaminergic neurons *in vitro*. In addition, some NTFs have been demonstrated to have effects on grafted fetal VM cells. For example, BDNF infusions to VM grafted rats have been shown to enhance striatal innervation (Yurek et al., 1996). In addition, bFGF infusions (Mayer et al., 1993b) or co-grafting of bFGF transfected fibroblasts (Takayama et al., 1995) have been shown to increase both survival and fiber outgrowth of transplanted DA neurons. GDNF has been shown to increase TH neuronal survival of in oculo grafts of fetal VM, suggesting that GDNF also may be able to enhance the survival of neurons grafted within the brain (Strömberg et al., 1993). As other factors have been shown to be trophic for dopaminergic neurons, investigation of additional co-grafting combinations are of interest. Moreover, characterization of the trophic mechanisms may indicate the underlying cause of the low survival rate.

The cause of the neuronal attrition is thought to be a result of oxidative stress associated with cell preparation and transplantation, and several studies support this hypothesis. Lazaroids are aminosteroids which inhibit the generation of free radicals and lipid peroxidation. Treatment of fetal VM cell cultures with lazarooids has been found to increase the survival of TH+ neurons (Frodl et al., 1994b). Moreover, lazarooid treatment of VM cells has been demonstrated to significantly increase cell viability prior to grafting and increase TH+ neuronal survival following transplantation by a factor of 2.6 (Nakao et al., 1994). Further evidence for the involvement of oxidative stress in the low survival rate of grafted dopaminergic neurons is provided by studies in which donor neurons were obtained from transgenic mice overexpressing SOD (Nakao et al., 1995). Similar to the survival effect observed in the lazarooid experiments,

grafting of tissue derived from the SOD transgenics resulted in improved cell viability and a 4-fold increase in TH+ neuronal survival. These results support the theory that the low yield of TH+ neurons is due at least in part to oxidative stress. Thus, administration of lazaroids or other antioxidative agents may be expected to improve the yield of surviving DA neurons in clinical transplantation as well.

Considerations in clinical neurotransplantation. While neurotransplantation of fetal dopaminergic neurons holds promise for the treatment of PD, parkinsonian symptoms are not completely alleviated by grafting (see Lindvall, 1991 for review). Similar to the observations from animal studies, the incomplete recovery is thought to be attributable to insufficient reinnervation, incomplete reciprocal connections with the host due to ectopic graft location, as well as a failure to compensate for the non-dopaminergic components of PD. In addition to the aforementioned limited survival and immunological issues, additional factors are relevant to clinical transplantation.

Unlike the toxic insults which acutely produce animal models, PD is a progressive disorder of nigral degeneration. It is therefore possible that ongoing disease progression could affect the grafted neurons (Lindvall, 1991). However, long-term graft survival with improved fluorodopa uptake has been observed, suggesting that the grafted neurons are not affected (Lindvall et al., 1994). In addition, patients receiving fetal grafts remain on L-dopa therapy. As L-dopa has been suggested to be toxic for dopaminergic neurons, there has been a theoretical concern that continued L-dopa therapy may negatively impact the grafted neurons. However, evaluation of rats receiving transplants and chronic

L-dopa/carbidopa treatment have failed to demonstrate any detrimental effect of L-dopa on graft function (Blunt et al., 1991a) or TH neuron survival and outgrowth (Blunt et al., 1991b).

The development of strategies to improve cell survival and the further refinement of grafting techniques and optimization of graft placement will likely improve the clinical outcome of PD patients receiving VM grafts.

Conclusions

Accumulating evidence indicates the involvement of oxidative stress and excitotoxicity in the pathogenesis of neurodegenerative disorders such as PD. As primarily one neuronal population is affected in PD, therapeutic approaches directed at protection of dopaminergic neurons may prevent disease progression and the onset of clinical symptoms. However, if cell death cannot be prevented, neuronal replacement and reconstruction of synaptic circuitry via the transplantation of fetal DA neurons is indeed possible.

NTFs are able to protect against cellular insults suggesting their potential clinical applicability. The pathway to neuronal death involves several mechanisms, including increased intracellular calcium and the generation of free radicals. Thus, NTF intervention at single or multiple sites in this process may protect against cellular insults and degenerative processes. By preventing the formation of oxidative radicals and/or enhancing radical scavenging capacities, NTFs may prevent neuronal damage.

The various mechanisms for protection coupled with the multiple sites for possible intervention suggest that NTFs acting via different cellular mechanisms or at sequential sites in these neurodegenerative pathways may provide additive or synergistic protective effects. While the precise mechanisms of NTF-mediated neuroprotection are not fully characterized, the trophic and protective effects of NTFs indicate their promise for future therapeutic application. However, the clinical utility of NTFs is presently limited by delivery and dosage issues. Strategies to circumvent the BBB need to be refined as systemic delivery of NTFs may be associated with untoward effects via actions on additional cell populations. Furthermore, the overlap in NTF specificity indicates that the delivered factor is likely to affect more than the target neuronal population. The evaluation of dose-response relationships and long-term effects of NTF treatment will aid in the development of NTFs as a therapeutic approach for the treatment of neurodegenerative disorders.

Presently, there are no neuroprotective therapies available for the treatment of PD. While current pharmacological therapies are limited by significant side effects, neurotransplantation of fetal dopaminergic cells provides a therapeutic alternative for PD. Extensive studies in rodents have demonstrated that grafted dopaminergic neurons can reinnervate the DA depleted striatum, mediate functional recovery, restore DA levels, and form synaptic connections with host neuronal circuitry. Importantly, grafted DA neurons exhibit regulatory capacities. The regulatory capacity and graft-host integration demonstrate the advantages of transplanted neurons compared to genetically engineered cells, which do not possess this ability. The long-term graft survival and functional

improvement observed in transplanted parkinsonian patients demonstrate the efficacy of this treatment approach.

The optimal treatment of neurodegenerative disorders would prevent ongoing neuronal death and halt disease progression. The present results support the idea that NTFs may be able to prevent ongoing neurodegenerative processes, perhaps by decreasing oxidative stress. While NTFs may provide such protection, further characterization of these factors is necessary prior to achieving clinical utility. While neuroprotective therapies remain experimental, reconstruction of neuronal circuitry may be achieved via neurotransplantation of fetal dopaminergic neurons. Current transplantation approaches are constrained in part by the limited supply of tissue. Yet, the continued refinement of methodologies as well as the development of alternate cell sources and strategies to improve cell survival will likely result in the greater accessibility of neurotransplantation as a treatment for PD.

REFERENCES

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* 94, 239-247.

Abrous, D. N., Shaltot, A. R. A., Torres, E. M., and Dunnett, S. B. (1993). Dopamine rich grafts in the neostriatum and/or nucleus accumbens: Effects on drug-induced behaviours and skilled paw-reaching. *Neuroscience* 53, 187-197.

Ahlskog, J. E. (1993). Cerebral transplantation for Parkinson's disease: Current progress and future prospects. *Mayo Clin. Proc.* 68, 578-591.

Albin, R. L., and Greenamyre, J. T. (1992). Alternative excitotoxic hypotheses. *Neurology* 42, 733-738.

Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends. Neurosci.* 12, 366-375.

Alexander, G. E., and Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: Neural substrates of parallel processing. *Trends in Neurosci.* 13, 266-271.

Altar, C. A., Boylan, C. B., Fritsche, M., Jackson, C., Hyman, C., and Lindsay, R. M. (1994a). The neurotrophins NT-4/5 and BDNF augment serotonin, dopamine, and GABAergic systems during behaviorally effective infusions to the substantia nigra. *Exp. Neurol.* 130, 31-40.

Altar, C. A., Boylan, C. B., Fritsche, M., Jones, B. E., Jackson, C., Wiegand, S. J., Lindsay, R. M., and Hyman, C. (1994b). Efficacy of brain-derived neurotrophic factor and neurotrophin-3 on neurochemical and behavioral deficits associated with partial nigrostriatal dopamine lesions. *J. Neurochem.* 63, 1021-1032.

Altar, C. A., Boylan, C. B., Jackson, C., Hershenson, S., Miller, J., Wiegand, S. J., Lindsay, R. M., and Hyman, C. (1992). Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. *Proc. Natl. Acad. Sci. USA* 89, 11347-11351.

Altar, C. A., Fritsche, M., Holst, P., and Radke, S. (1995). Presence of BDNF protein in brain and plasma detected by a sensitive and specific enzyme-linked immunoassay. *Soc. Neurosci. Abstr.* 21, in press.

Altar, C. A., Siuciak, J. A., Wright, P., Ip, N. Y., Lindsay, R. M., and Wiegand, S. J. (1994c). In situ hybridization of *trkB* and *trkC* receptor mRNA in rat forebrain and association with high-affinity binding of [¹²⁵I]BDNF, [¹²⁵I]NT-4/5, and [¹²⁵I]NT-3. *Eur. J. Neurosci.* 6, 1389-1405.

Ambani, L. M., Van Woert, M. H., and Murphy, S. (1975). Brain peroxidase and catalase in Parkinson disease. *Arch. Neurol.* 32, 114-118.

Anderson, K. J., Dam, D., Lee, S., and Cotman, C. W. (1988). Basic fibroblast growth factor prevents death of lesioned cholinergic neurons in vivo. *Nature* 332, 360-361.

Appel, S. H. (1981). A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and alzheimer disease. *Ann. Neurol.* 10, 499-505.

Baird, A. (1994). Fibroblast growth factors: activities and significance of non-neurotrophin neurotrophic growth factors. *Curr. Opin. Neurobiol.* 4, 78-86.

Bal, A., Chritin, M., Mennicken, F., Abrous, D. N., LeMoal, M., Feuerstein, C., and Herman, J. P. (1993). Transplantation of fetal nigral cells reverses the increase of preproenkephalin mRNA levels in the rat striatum caused by 6-OHDA lesion of the dopaminergic nigrostriatal pathway: a quantitative in situ hybridization study. *Mol. Brain Res.* 18, 221-227.

Bandmann, O., Davis, M. B., Marsden, C. D., and Harding, A. E. (1995). Sequence of the superoxide dismutase 1 (SOD1) gene in familial Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 59, 90-91.

Barde, Y.-A., Edgar, D., and Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* 1, 549-553.

Barde, Y. A. (1988). What, if anything, is a neurotrophic factor? *Trends Neurosci* 11, 343-6.

Bartlett, P. F., Rosenfeld, J., Bailey, K. A., Cheesman, H., Harvey, A. R., and Kerr, R. S. C. (1990). Allograft rejection overcome by immunoselection of neuronal precursor cells. *Prog. Brain Res.* 82, 153-.

Baumann, R. J., Jameson, H. D., McKean, H. E., Haack, D. G., and Weisberg, L. M. (1980). Cigarette smoking and Parkinson's disease: 1. A comparison of cases with matched neighbors. *Neurology* 30, 839-843.

Beal, M. F. (1992). Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann. Neurol.* 31, 119-130.

Beal, M. F. (1995). Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann. Neurol.* 38, 357-366.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., Storey, E., Srivastava, R., Rosen, B. R., and Hyman, B. T. (1993). Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *J. Neurosci.* 13, 4181-4192.

Beal, M. F., Ferrante, R. J., Henshaw, R., Matthews, R. T., Chan, P. H., Kowall, N. W., Epstein, C. J., and Schulz, J. B. (1995). 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgeneic mice. *J. Neurochem.* 65, 919-922.

Beal, M. F., Matson, W. R., Swartz, K. J., Gamache, P. H., and Bird, E. D. (1990). Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. *J Neurochem* 55, 1327-1339.

Beck, K. D., Knusel, B., Winslow, J. W., Rosenthal, A., Burton, L. E., Nikolics, K., and Hefti, F. (1992). Pretreatment of dopaminergic neurons in culture with brain-derived neurotrophic factor attenuates toxicity of 1-methyl-4-phenylpyridinium. *Neurodegeneration* 1, 27-36.

Beck, K. D., Valverde, J., Alexi, T., Poulsen, K., Moffat, B., Vandlen, R. A., Rosenthal, A., and Hefti, F. (1995). Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. *Nature* 373, 339-341.

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., and Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci., USA* 87, 1620-1624.

Benabid, A. L., Pollak, P., Gervason, C., Hoffmann, D., Gao, D. M., Hommel, M., Perret, J. E., and DeRougemont, J. (1991). Long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *Lancet* 337, 403-406.

Berkemeier, L. R., Winslow, J. W., Kaplan, D. R., Nikolics, K., Goeddel, D. V., and Rosenthal, A. (1991). Neurotrophin-5: A novel neurotrophic factor that activates trk and trkB. *Neuron* 7, 857-866.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., and Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington: Clinical, morphological, and neurochemical correlations. *J. Neurol. Sci.* 20, 415-455.

Bierer, B. E., Holländer, G., Fruman, D., and Burakoff, S. J. (1993). Cyclosporin A and FK506: molecular mechanisms of immunosuppression and probes for transplantation biology. *Curr. Opin. Neurobiol.* 5, 763-773.

Bird, E. D., MacKay, A. V. P., Rayner, C. N., and Iversen, L. L. (1973). Reduced glutamic acid decarboxylase activity of post-mortem brain in Huntington's chorea. *Lancet* 1, 1090-1092.

Björklund, A. (1992). Dopaminergic transplants in experimental parkinsonism: cellular mechanisms of graft-induced functional recovery. *Curr. Opin. Neurobiol.* 2, 683-689.

Björklund, A. (1993). Better cells for brain repair. *Nature* 362, 414-415.

Björklund, A., Dunnett, S. B., Stenevi, U., Lewis, M. E., and Iversen, S. D. (1980a). Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. *Brain Res.* 199, 307-333.

Björklund, A., Schmidt, R. H., and Stenevi, U. (1980b). Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra. *Cell Tissue Res.* 212, 39-45.

Björklund, A., and Stenevi, U. (1979). Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res.* 177, 555-560.

Björklund, A., Stenevi, U., Dunnett, S. B., and Gage, F. H. (1982). Cross-species neural grafting in a rat model of Parkinson's disease. *Nature* 298, 652-654.

Björklund, A., Stenevi, U., Schmidt, R. H., Dunnett, S. B., and Gage, F. H. (1983a). Intracerebral grafting of neuronal cell suspensions I. Introduction and general methods of preparation. *Acta Physiol. Scand.*, Suppl. 522, 1-7.

Björklund, A., Stenevi, U., Schmidt, R. H., Dunnett, S. B., and Gage, F. H. (1983b). Intracerebral grafting of neuronal cell suspensions II. survival and growth of nigral cell suspensions implanted in different brain sites. *Acta Physiol. Scand.* Suppl. 522, 9-18.

Björklund, A., Stenevi, U., and Svendgaard, N.-A. (1976). Growth of transplanted monoaminergic neurons into the adult hippocampus along the perforant path. *Nature* 262, 787-790.

Blunt, S., Jenner, P., and Marsden, C. D. (1991a). The effect of chronic L-dopa treatment on the recovery of motor function in 6-hydroxydopamine-lesioned rats receiving ventral mesencephalic grafts. *Neuroscience* 40, 453-464.

Blunt, S. B., Jenner, P., and Marsden, C. D. (1991b). The effect of L-dopa and carbidopa treatment on the survival of rat fetal dopamine grafts assessed by tyrosine hydroxylase immunohistochemistry and [3H]mazindol autoradiography. *Neuroscience* 43, 95-110.

Bolam, J. P., Freund, T. F., Björklund, A., Dunnett, S. B., and Smith, A. D. (1987). Synaptic input and local output of dopaminergic neurons in grafts that functionally reinnervate the host neostriatum. *Exp. Brain Res.* 69, 131-146.

Bowenkamp, K. E., Hoffman, A. F., Gerhardt, G. A., Henry, M. A., Biddle, P. T., Hoffer, B. J., and Granholm, A.-C. E. (1995). Glial cell-line derived neurotrophic factor supports survival of injured midbrain dopaminergic neurons. *J. Comp. Neurol.* 355, 479-489.

Breakefield, X. O., and DeLuca, N. A. (1991). Herpes simplex for gene delivery to neurons. *New Biologist* 3, 203-218.

Brennan, W. A., Bird, E. D., and Aprille, J. R. (1985). Regional mitochondrial respiratory activity in Huntington's disease brain. *J. Neurochem.* 44, 1948-1950.

Broadwell, R. D., Charlton, H. M., Balin, B. J., and Salcman, M. (1987). Angioarchitecture of the CNS, pituitary gland, and intracerebral grafts revealed with peroxidase cytochemistry. *J. Comp. Neurol.* 260, 47-62.

Brooks, D. J. (1991). Detection of preclinical Parkinson's disease with PET. *Neurology* 41S, 24-27.

Brouillet, E., Hantraye, P., Ferrante, R. J., Dolan, R., Leroy-Willing, A., Kowall, N. W., and Beal, M. F. (1995). Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc. Natl. Acad. Sci., USA* 92, 7105-7109.

Brundin, P., Barbin, G., Isacson, O., Mallet, M., Chamak, B., Prochiantz, A., Gage, F. H., and Björklund, A. (1985a). Survival of intracerebrally grafted rat dopamine neurons previously cultured in vitro. *Neurosci. Lett.* 61, 79-84.

Brundin, P., Barbin, G., Strecker, R. E., Isacson, O., Prochiantz, A., and Björklund, A. (1988a). Survival and function of dissociated rat dopamine neurones grafted at different developmental stages or after being cultured in vitro. *Dev. Brain Res.* 39, 233-243.

Brundin, P., Isacson, O., and Björklund, A. (1985b). Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. *Brain Res.* 331, 251-259.

Brundin, P., Nilsson, O. G., Gage, F. H., and Björklund, A. (1985c). Cyclosporin A increases survival of cross-species intrastriatal grafts of embryonic dopamine containing neurons. *Exp. Brain Res.* 60, 204-208.

Brundin, P., Nilsson, O. G., Strecker, R. E., Lindvall, O., Astedt, B., and Björklund, A. (1986). Behavioral effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. *Exp. Brain Res.* 65, 235-240.

Brundin, P., Strecker, R. E., Londos, E., and Björklund, A. (1987). Dopamine neurons grafted unilaterally to the nucleus accumbens affect drug-induced circling and locomotion. *Exp. Brain Res.* 69, 183-194.

Brundin, P., Strecker, R. E., Widner, H., Clarke, D. J., Nilsson, O. G., Åstedt, B., Lindvall, O., and Björklund, A. (1988b). Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: immunological aspects, spontaneous and drug-induced behavior, and dopamine release. *Exp. Brain Res.* 70, 192-208.

Brundin, P., Widner, H., Nilsson, O. G., Strecker, R. E., and Björklund, A. (1989). Intracerebral xenografts of dopamine neurons: the role of immunosuppression and the blood-brain barrier. *Exp. Brain Res.* 75, 195-207.

Burn, D. J., Mark, M. H., Playford, E. D., Maraganore, D. M., Zimmerman, T. R., Duvoisin, R. C., Harding, A. E., Marsden, C. D., and Brooks, D. J. (1992).

Parkinson's disease in twins studied with F-dopa and positron emission tomography. *Neurology* 42, 1894-1900.

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., and Kopin, I. J. (1983). A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* 80, 4546-4550.

Calne, D. B. (1993). Treatment of Parkinson's disease. *N. Engl. J. Med.* 329, 1021-1027.

Calne, D. B., and Langston, J. W. (1983). Aetiology of Parkinson's disease. *Lancet* ii, 1457-1459.

Campbell, K. J., Takada, M., and Hattori, T. (1990). Evidence for retrograde axonal transport of MPP⁺ in the rat. *Neurosci. Lett.* 118, 151-154.

Carrero-Valenzuela, R., Linblad, K., Payami, H., Johnson, W., Schalling, M., Stenroos, E. S., Shattuc, S., Nutt, J., Brice, A., and Litt, M. (1995). No evidence for association of familial Parkinson's disease with CAG repeat expansion. *Neurology* 45, 1760-1763.

Cenci, M. A., Campbell, K., and Björklund, A. (1993). Neuropeptide messenger RNA expression in the 6-hydroxydopamine-lesioned rat striatum reinnervated by fetal dopaminergic transplants: Differential effects of the grafts on preproenkephalin, preprotachykinin and prodynorphin messenger RNA levels. *Neuroscience* 57, 275-296.

Cenci, M. A., Kalén, P., Mandel, R. J., Wictorin, K., and Björklund, A. (1992). Dopaminergic transplants normalize amphetamine- and apomorphine-induced fos expression in the 6-hydroxydopamine lesioned striatum. *Neuroscience* 46, 943-957.

Chan, P., DeLanney, L. E., Irwin, I., Langston, J. W., and DiMonte, D. (1991). Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse brain. *J. Neurochem.* 57, 348-351.

Chase, T. N., Mouradian, M. M., and Engber, T. M. (1993). Motor response complications and the function of striatal efferent systems. *Neurology* 43S, S23-S27.

Cheng, B., and Mattson, M. P. (1991). NGF and bFGF protect rat hippocampal and human cortical neurons against hypoglycemic damage by stabilizing calcium homeostasis. *Neuron* 7, 1031-1041.

Chirgwin, J. M., Przybyla, A. E., Macdonald, R. J., and Rutter, W. J. (1979).

Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18, 5294-5299.

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system.

Neuron 1, 623-634.

Choi, D. W. (1992). Excitotoxic cell death. *J. Neurobiol.* 23, 1261-1276.

Choi-Lundberg, D. L., and Bohn, M. C. (1995). Ontogeny and distribution of glial cell-line derived neurotrophic factor (GDNF) mRNA in rat. *Dev. Brain Res.* 85, 80-88.

Clarke, D. J., Brundin, P., Strecker, R. E., Nilsson, O. G., Björklund, A., and Lindvall, O. (1988). Human fetal dopamine neurons grafted in a rat model of Parkinson's disease: ultrastructural evidence for synapse formation using tyrosine hydroxylase immunocytochemistry. *Exp. Brain Res.* 73, 115-126.

Clough, C. G. (1991). Parkinson's disease: management. *Lancet* 337, 1324-1327.

Cohen, G., and Heikkila, R. E. (1974). The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J. Biol. Chem.* 249, 2447-2452.

Collier, T. J., Redmond, D. E., Sladek, C. D., Gallagher, M. J., Roth, R. H., and Sladek, J. R. (1987). Intracerebral grafting and culture of cryopreserved primate dopamine neurons. *Brain Res.* 436, 363-366.

Cordon-Cardo, C., Tapley, P., Jing, S., Nanduri, V., O'Rourke, E., Lamballe, F., Kovary, K., Klein, R., Jones, K. R., Reichardt, L. F., and Barbacid, M. (1991). The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. *Cell* 66, 173-183.

Cotzias, G. C., Papavasiliou, P. S., and Gellene, R. (1969). Modification of parkinsonism-chronic treatment with L-dopa. *N. Engl. J. Med.* 280, 337-345.

Daniloff, J. K., Low, W. C., Bodony, R. P., and Wells, J. (1985). Cross-species neural transplants of embryonic septal nuclei to the hippocampal formation of adult rats. *Exp. Brain Res.* 59, 73-82.

Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N. Y., and Yancopoulos, G. D. (1993). LIFR β and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. *Science* 260, 1805-1808.

Davis, S., Aldrich, T. H., Valenzuela, D. M., Wong, V., Furth, M. E., Squinto, S. P., and Yancopoulos, G. D. (1991). The receptor for ciliary neurotrophic factor. *Science* 253, 59-63.

Davis, S., and Yancopoulos, G. D. (1993). The molecular biology of the CNTF receptor. *Curr. Opin. Neurobiol.* 3, 20-24.

Dawbarn, D., De Quidt, M. E., and Emson, P. C. (1985). Survival of basal ganglia neuropeptide Y-somatostatin neurones in Huntington's disease. *Brain Res.* 340, 251-260.

Dawson, T. M., Dawson, V. L., Gage, F. H., Fisher, L. J., Hunt, M. A., and Wamsley, J. K. (1991a). Downregulation of muscarinic receptors in the rat caudate-putamen after lesioning of the ipsilateral nigrostriatal pathway with 6-hydroxydopamine (6-OHDA): normalization by fetal mesencephalic transplants.

Dawson, T. M., Dawson, V. L., Gage, F. H., Fisher, L. J., Hunt, M. A., and Wamsley, J. K. (1991b). Functional recovery of supersensitive dopamine receptors after intrastriatal grafts of fetal substantia nigra. *Exp. Neurol.* 111, 282-292.

Dawson, T. M., Steiner, J. P., Dawson, V. L., Dinerman, J. L., Uhl, G. R., and Snyder, S. H. (1993). Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc. Natl. Acad. Sci., USA* 90, 9808-9812.

Dawson, V. L., Dawson, T. M., London, E. D., Brecht, D. S., and Snyder, S. H. (1991c). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl. Acad. Sci., USA* 88, 6368-6371.

Deacon, T. W., Pakzaban, P., Burns, L. H., Dinsmore, J., and Isacson, O. (1994). Cytoarchitectonic development, axon-glia relationships, and long distance axon growth of porcine striatal xenografts in rats. *Exp. Neurol.* 130, 151-167.

DeLong, M. (1990). Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 13, 281-285.

Dexter, D., Carter, C., Agid, F., Agid, Y., Lees, A. J., Jenner, P., and Marsden, C. D. (1986). Lipid peroxidation as cause of nigral cell death in Parkinson's disease. *Lancet ii*, 639-640.

Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., and Marsden, C. D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* 52, 381-389.

Dexter, D. T., Holley, A. E., Flitter, W. D., Slater, T. F., Wells, F. R., Daniel, S. E., Lees, A. J., Jenner, P., and Marsden, C. D. (1994). Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: an HPLC and ESR study. *Mov. Disord.* 9, 92-97.

Dexter, D. T., Wells, F. R., Agid, F., Agid, Y., Lees, A. J., Jenner, P., and Marsden, C. D. (1987). Increased nigral iron content in postmortem parkinsonian brain. *Lancet ii*, 1219-1220.

Dickerson, J. W. T., and Dobbing, J. (1967). Prenatal and postnatal growth and development of the central nervous system of the pig. *Proc. Roy. Soc. Brit.* 166, 384-395.

Doucet, G., Murata, Y., Brundin, P., Bosler, O., Mons, N., Geffard, M., Ouimet, C. C., and Björklund, A. (1989). Host afferents into intrastriatal transplants of fetal ventral mesencephalon. *Exp. Neurol.* 106, 1-19.

Duan, W.-M., Brundin, P., Grasbon-Frodl, E. M., and Widner, H. (1996). Methylprednisolone prevents rejection of intrastriatal grafts of xenogeneic embryonic neural tissue in adult rats. *Brain Res.* 712, 199-212.

Duan, W.-M., Widner, H., Björklund, A., and Brundin, P. (1993). Sequential intrastriatal grafting of allogeneic embryonic dopamine-rich neuronal tissue in adult rats: Will the second graft be rejected. *Neuroscience* 57, 261-274.

Duan, W.-M., Widner, H., and Brundin, P. (1995a). Temporal pattern of host responses against intrastriatal grafts of syngeneic, allogeneic or xenogeneic embryonic neuronal tissue in rats. *Exp. Brain Res.* 104, 227-242.

Duan, W.-M., Widner, H., Frodl, E. M., and Brundin, P. (1995b). Immune reactions following systemic immunization prior or subsequent to intrastriatal transplantation of allogeneic mesencephalic tissue in adult rats. *Neuroscience* 64, 629-641.

Dunnett, S. B., Björklund, A., Schmidt, R. H., Stenevi, U., and Iversen, S. D. (1983). Intracerebral grafting of neuronal cell suspensions IV. Behavioral recovery in rats with unilateral 6-OHDA lesions following implantation of nigral cell suspensions in different forebrain sites. *Acta Physiol. Scand.*, Suppl. 522, 29-37.

Dunnett, S. B., Björklund, A., Stenevi, U., and Iversen, S. D. (1981a). Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Unilateral lesions. *Brain Res.* 215, 147-161.

Dunnett, S. B., Björklund, A., Stenevi, U., and Iversen, S. D. (1981b). Grafts of embryonic substantia nigra reinnervating the ventrolateral striatum ameliorate sensorimotor impairments and akinesia in rats with 6-OHDA lesions of the nigrostriatal pathway. *Brain Res.* 229, 209-217.

Dunnett, S. B., Hernandez, T. D., Summerfield, A., Jones, G. H., and Arbuthnott, G. (1988). Graft-derived recovery from 6-OHDA lesions: specificity of ventral mesencephalic graft tissues. *Exp. Brain Res.* 71, 411-424.

Dunnett, S. B., Wishaw, I. Q., Rogers, D. C., and H., J. G. (1987). Dopamine-rich grafts ameliorate whole body motor asymmetry and sensory neglect but not independent skilled limb use in rats with 6-hydroxydopamine lesions. *Brain Res.* 415, 63-78.

During, M. J., Naegle, J. R., O'Malley, K. L., and Geller, A. I. (1994). Long-term behavioral recovery in parkinsonian rats by HSV vector expressing tyrosine hydroxylase. *Science* 266, 1399-1402.

Duvoisin, R. C. (1986). On heredity, twins, and Parkinson's disease. *Ann. Neurol.* 19, 409-411.

Emerich, D. F., Hammang, J. P., Baetge, E. E., and Winn, S. R. (1994). Implantation of polymer-encapsulated human nerve growth factor-secreting fibroblasts attenuates the behavioral and neuropathological consequences of quinolinic acid injections in rodent striatum. *Exp. Neurol.* 130, 141-150.

Engel, J., and Bohn, M. C. (1991). The neurotrophic effects of fibroblast growth factors on dopaminergic neurons in vitro are mediated by mesencephalic glia. *J. Neurosci.* 11, 3070-3078.

Ernfors, P., Ibanez, C. F., Ebendal, T., Olson, L., and Persson, H. (1990). Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. *Proc. Natl. Acad. Sci., USA* 87, 5454-5458.

Fahn, S. (1974). "On-off" phenomenon with levodopa therapy in parkinsonism. *Neurology* 24, 431-441.

Fahn, S., and Cohen, G. (1992). The oxidant stress hypothesis in parkinson's disease: Evidence supporting it. *Ann. Neurol.* 32, 804-812.

Fearnley, J., and Lees, A. (1994). Pathology of parkinson's disease. In *Neurodegenerative diseases*, D. B. Calne, ed.: W. B. Saunders, Co.), pp. 545-554.

Ferrante, R. J., Beal, M. F., Kowall, N. W., Richardson Jr., E. P., and Martin, J. B. (1987). Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. *Brain Res.* 411, 162-166.

Ferrante, R. J., Kowall, N. W., Beal, M. F., Richardson Jr., E. P., Bird, E. D., and Martin, J. B. (1985). Selective sparing of a class of striatal neurons in Huntington's disease. *Science* 230, 561-563.

Finsen, B., Oteruelo, F., and Zimmer, J. (1988a). Immunocytochemical characterization of the cellular immune response to intracerebral xenografts of brain tissue. *Prog. Brain Res.* 78, 261-270.

Finsen, B., Poulsen, P. H., and Zimmer, J. (1988b). Xenografting of fetal mouse hippocampal tissue to the brain of adult rats: effects of Cyclosporin A treatment. *Exp. Brain Res.* 70, 117-133.

Finsen, B. R., Pedersen, E. B., Sorensen, T., Hokland, M., and Zimmer, J. (1990). Immune reactions against intracerebral murine xenografts of fetal hippocampal tissue and cultured cortical astrocytes in the adult rat. *Prog. Brain Res.* 82, 111-128.

Finsen, B. R., Sorensen, T., Castellano, B., Pedersen, E. B., and Zimmer, J. (1991). Leukocyte infiltration and glial reactions in xenografts of mouse brain tissue undergoing rejection in the adult rat brain. A light and electron microscopical immunocytochemical study. *J. Neuroimmunol.* 32, 159-183.

Fischer, W., Wictorin, K., Bjorklund, A., Williams, L. R., Varon, S., and Gage, F. H. (1987). Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature* 329, 65-68.

Fisher, L. J., Jinnah, H. A., Kale, L. C., Higgins, G. A., and Gage, F. H. (1991). Survival and function of intrastrially grafted primary fibroblasts genetically modified to produce l-dopa. *Neuron* 6, 371-380.

Fowler, J. S., Volkow, N. D., Wang, G.-J., Pappas, N., Logan, J., MacGregor, R., Alexoff, D., Shea, C., Schyler, D., Wolf, A. P., Warner, D., Zezulkova, I., and Cilento, R. (1996). Inhibition of monoamine oxidase B in the brains of smokers. *Nature* 379.

Freed, C. R., Breeze, R. E., Rosenberg, N. L., Schneck, S. A., Kriek, E., Qi, J.-X., Lone, T., Zhang, Y.-B., Snyder, J. A., Wells, T. H., Ramig, L. O., Thompson, L., Mazziotta, J. C., Huang, S. C., Grafton, S. T., Brooks, D., Sawle, G., Schroter, G., and Ansari, A. A. (1992). Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N. Engl. J. Med.* 327, 1549-1555.

Freed, C. R., Breeze, R. E., Rosenberg, N. L., Schneck, S. A., Wells, T. H., Barrett, J. N., Grafton, S. T., Huang, S. C., Eidelberg, D., and Rottenberg, D. A. (1990a). Transplantation of human fetal dopamine cells for Parkinson's disease. Results at one year. *Arch. Neurol.* 47, 505-512.

Freed, W. J., Dymecki, J., Poltorak, M., and Rodgers, C. R. (1988). Intraventricular brain allografts and xenografts: studies of survival and rejection with and without systemic sensitization. *Prog. Brain Res.* 78, 233-241.

Freed, W. J., Ko, G. N., Niehoff, D. L., Kuhar, M. J., Hoffer, B. J., Olson, L., Cannon-Spoor, H. E., Morihisa, J. M., and Wyatt, R. J. (1983). Normalization of spiroperidol binding in the denervated rat striatum by homologous grafts of substantia nigra. *Science* 222, 937-939.

Freed, W. J., Perlow, M. J., Karoum, F., Seiger, A., Olson, L., Hoffer, B. J., and Wyatt, R. J. (1980). Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: long-term behavioral, biochemical, and histochemical studies. *Ann. Neurol.* 8, 510-519.

Freed, W. J., Poltorak, M., and Becker, J. B. (1990b). Intracerebral adrenal medulla grafts: a review. *Exp. Neurol.* 110, 139-166.

Freeman, T. B., Olanow, C. W., Hauser, R. A., Nauert, G. M., Smith, D. A., Borlongan, C. V., Sanberg, P. R., Holt, D. A., Kordower, J. H., Vingerhoets, F. J. G., Snow, B. J., Calne, D., and Gauger, L. L. (1995). Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Exp. Neurol.* 38, 379-388.

Freeman, T. B., Wojak, J. C., Brandeis, L., Michel, J. P., Pearson, J., and Flamm, E. S. (1988). Cross-species intracerebral grafting of embryonic swine dopaminergic neurons. *Prog. Brain Res.* 78, 473-477.

Freund, T. F., Bolam, J. P., Björklund, A., Stenevi, U., Dunnett, S. B., Powell, J. F., and Smith, A. D. (1985). Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum: a tyrosine hydroxylase immunocytochemical study. *J. Neurosci.* 5, 603-616.

Friden, P. M., Walus, L. R., Watson, P., Doctrow, S. R., Kozarich, J. W., Bäckman, C., Bergman, H., Hoffer, B., Bloom, F., and Granholm, A.-C. (1993). Blood-brain barrier penetration and in vivo activity of an NGF conjugate. *Science* 259, 373-377.

Frim, D. M., Emanuel, R. L., Robinson, B. G., Smas, C. M., Adler, G. K., and Majzoub, J. A. (1988). Characterization and gestational regulation of corticotropin-releasing hormone and messenger RNA in the human placenta. *J. Clin. Invest.* 82, 293-299.

Frim, D. M., Short, M. P., Breakefield, X. O., and Isacson, O. (1993a). Biological gene-product delivery to the brain: a protocol for retroviral gene transfer into cultured cells and intracerebral transplantation. *Neuroprotocols: a companion to methods in neurosciences* 3, 63-68.

Frim, D. M., Short, M. P., Rosenberg, W. S., Simpson, J., Breakefield, X. O., and Isacson, O. (1993b). Local protective effects of nerve growth factor-secreting fibroblasts against excitotoxic lesions in the rat striatum. *J. Neurosurg.* 78, 267-273.

Frim, D. M., Simpson, J., Uhler, T. A., Short, M. P., Bossi, S. R., Breakefield, X. O., and Isacson, O. (1993c). Striatal degeneration induced by mitochondrial blockade is prevented by biologically delivered NGF. *J. Neurosci. Res.* 35, 452-458.

Frim, D. M., Uhler, T. A., Galpern, W. R., Beal, M. F., Breakefield, X. O., and Isacson, O. (1994a). Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. *Proc. Natl. Acad. Sci. USA* 91, 5104-5108.

Frim, D. M., Uhler, T. A., Short, M. P., Ezzedine, Z. D., Klagsbrun, M., Breakefield, X. O., and Isacson, O. (1993d). Effects of biologically delivered NGF, BDNF, and bFGF in striatal excitotoxic lesions. *Neuroreport* 4, 367-370.

Frim, D. M., Wüllner, U., Beal, M. F., and Isacson, O. (1994b). Implanted NGF-producing fibroblasts induce catalase and modify ATP levels but do not affect glutamate receptor binding or NMDA receptor expression in the rat striatum. *Exp. Neurol.* 128, 172-180.

Frodl, E. M., Duan, W.-M., Sauer, H., Kupsch, A., and Brundin, P. (1994a). Human embryonic dopamine neurons xenografted to the rat: effects of cryopreservation and varying regional source of donor cells on transplant survival, morphology, and function. *Brain Res.* 647, 286-298.

Frodl, E. M., Nakao, N., and Brundin, P. (1994b). Lazaroids improve the survival of cultured rat embryonic mesencephalic neurones. *Neuroreport* 5, 2393-2396.

Gage, F. H., Wolff, J. A., Rosenberg, M. B., Xu, L., Yee, J.-K., Shults, C., and Friedmann, T. (1987). Grafting genetically modified cells to the brain: possibilities for the future. *Neuroscience* 23, 795-807.

Gall, C. M., Gold, S. J., Isackson, P. J., and Seroogy, K. B. (1992). Brain-derived neurotrophic factor and neurotrophin-3 mRNAs are expressed in ventral midbrain regions containing dopaminergic neurons. *Mol. Cell. Neurosci.* 3, 56-63.

Garthwaite, J., Charles, S. L., and Chess-Williams, R. (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests a role as intracellular messenger in the brain. *Nature* 336, 385-388.

Gash, D. M., Zhang, Z., Ovadia, A., Cass, W. A., Yi, A., Simmerman, L., Russell, D., Martin, D., Lapchak, P. A., Collins, F., Hoffer, B. J., and Gerhardt, G. A. (1996). Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* 380, 252-255.

Gasser, T., Wszolek, K., Trofatter, J., Ozelius, L., Uitti, R. J., Lee, C. S., Gusella, J., Pfeiffer, R. F., Calne, D. B., and Breakefield, X. O. (1994). Genetic linkage studies in autosomal dominant parkinsonism: Evaluation of seven candidate genes. *Ann. Neurol.* 36, 387-396.

Godwin-Austen, R. B., Lee, P. N., Marmot, M. G., and Stern, G. M. (1982). Smoking in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 45, 577-581.

Goetz, C. G., DeLong, M. R., Penn, R. D., and Bakay, R. A. E. (1993). Neurosurgical horizons in Parkinson's disease. *Neurology* 43, 1-7.

Goetz, C. G., Olanow, C. W., Koller, W. C., Penn, R. D., Cahill, D., Morantz, R., Stebbins, G., Tanner, C. M., Klawans, H. L., Shannon, K. M., Comella, C. L., Witt, T., Cox, C., Waxman, M., and Gauger, L. (1989). Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. *N. Engl. J. Med.* 320, 337-341.

Goetz, C. G., Stebbins, G. T., Klawans, H. L., Koller, W. C., Grossman, R. G., Bakay, R. A. E., Penn, R. D., and Registry, U. P. F. N. T. (1991). United parkinson foundation neurotransplantation registry on adrenal medullary transplants: Presurgical, and 1- and 2- year follow-up. *Neurology* 41, 1719-1722.

Golbe, L. I. (1990). The genetics of Parkinson's disease: A reconsideration. *Neurology* 40S, 7-14.

Golbe, L. I., Cody, R. A., and Duvoisin, R. C. (1986). Smoking and Parkinson's disease-search for a dose-response relationship. *Arch. Neurol.* 43, 774-778.

Golbe, L. I., Di Iorio, G., Bonavita, V., Miller, D. C., and Duvoisin, R. C. (1990). A large kindred with autosomal dominant Parkinson's disease. *Ann. Neurol.* 27, 276-282.

Gonzalez, A. M., Berry, M., Maher, P. A., Logan, A., and Baird, A. (1995). A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the adult brain. *Brain Res.* 701, 201-226.

Gossen, M., and Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci., USA* 89, 5547-5551.

Graveland, G. A., Williams, R. S., and DiFiglia, M. (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science* 227, 770-773.

Greenamyre, J. T., Penney, J. B., Young, A. B., D'Amato, C., Hicks, S. P., and Shoulson, I. (1985). Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. *Science* 227, 1496-1499.

Haas, R. H., Nasirian, F., Nakano, K., Ward, D., Pay, M., Hill, R., and Shults, C. W. (1995). Low platelet mitochondrial complex I and Complex II/III activity in early untreated Parkinson's disease. *Ann. Neurol.* 37, 714-722.

Hagg, T. (1994). Neurotrophins (BDNF, NT-3, NT-4) prevent death of axotomized adult rat substantia nigra neurons. *Soc. Neurosci. Abstr.* 20, 441.

Hagg, T., Manthorpe, M., Vahlsing, H. L., and Varon, S. (1988). Delayed treatment with nerve growth factor reverses the apparent loss of cholinergic neurons after acute brain damage. *Exp. Neurol.* 101, 303-312.

Hagg, T., and Varon, S. (1993). Ciliary neurotrophic factor prevents degeneration of adult rat substantia nigra dopaminergic neurons in vivo. *Proc. Natl. Acad. Sci. USA* 90, 6315-6319.

Hallbook, F., Ibanez, C. F., and Persson, H. (1991). Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* 6, 845-858.

Hauser, R. A., Freeman, T. B., and Olanow, C. W. (1995). Surgical therapies for parkinson's disease. In *Treatment of Movement Disorders*, R. Kurlan, ed.: J. B. Lippincott Co.), pp. 57-91.

Hefti, F. (1983). Is Alzheimer's disease caused by a lack of nerve growth factor? *Ann. Neurol.* 13, 109-110.

Hefti, F. (1986). Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.* 6, 2155-2162.

Hefti, F. (1994). Neurotrophic factor therapy for nervous system degenerative diseases. *J. Neurobiol.* 25, 1418-1435.

Hefti, F., and Melamed, E. (1980). L-DOPA's mechanism of action in Parkinson's disease. *Trends Neurosci.* 3, 229-231.

Heikkila, R., and Cohen, G. (1971). Inhibition of biogenic amine uptake by hydrogen peroxide: A mechanism for toxic effects of 6-hydroxydopamine. *Science* 172, 1257-1258.

Heikkila, R. E., Hess, A., and Duvoisin, R. C. (1984a). Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science* 224, 1451-1453.

Heikkila, R. E., Manzino, L., Cabbat, F. S., and Duvoisin, R. C. (1984b). Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature* 311, 467-9.

Heikkila, R. E., Nicklas, W. J., and Duvoisin, R. C. (1985). Dopaminergic toxicity after the stereotaxic administration of the 1-methyl-4-phenylpyridinium ion (MPP+) to rats. *Neurosci. Lett.* 59, 135-140.

Herkenham, M., Little, M. D., Bankiewicz, K., Yang, S.-C., Markey, S. P., and Johannessen, J. N. (1991). Selective retention of MPP+ within the monoaminergic systems of the primate brain following MPTP administration: an in vivo autoradiographic study. *Neuroscience* 40, 135-158.

Hoehn, M. M., and Yahr, M. D. (1967). Parkinsonism: Onset, progression and mortality. *Neurology* 17, 427-442.

Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J., and Barde, Y.-A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO* 9, 2459-2464.

Hofer, M. M., and Barde, Y.-A. (1988). Brain-derived neurotrophic factor prevents neuronal death in vivo. *Nature* 331, 261-262.

Hoffer, B. J., Hoffman, A., Bowenkamp, K., Huettl, P., Hudson, J., Martin, D., Lin, L.-F. H., and Gerhardt, G. A. (1994). Glial cell-line derived neurotrophic factor reverses toxin-induced injury to midbrain dopaminergic neurons in vivo. *Neurosci. Lett.*, 107-111.

Hohn, A., Leibrock, J., Bailey, K., and Barde, Y.-A. (1990). Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* 344, 339-341.

Holtzman, D. M., Kilbridge, J., Li, Y., Cunningham, E. T., Lenn, N. J., Clary, D. O., Reichardt, L. F., and Mobley, W., C. (1995). TrkA expression in the CNS: evidence for the existence of several novel NGF-responsive neurons. *J. Neurosci.* 15, 1567-1576.

Honey, C. R., Clarke, D. J., Dallman, M. J., and Charlton, H. M. (1990). Human neural graft function in rats treated with anti-interleukin II antibody. *Neuroreport* 1, 247-249.

Horellou, P., Brundin, P., Kalén, P., Mallet, J., and Björklund, A. (1990). In vivo release of DOPA and dopamine from genetically engineered cells grafted to the denervated striatum. *Neuron* 5, 393-402.

Horellou, P., Vigne, E., Castel, M.-N., Barnéoud, P., Colin, P., Perricaudet, M., Delaère, P., and Mallet, J. (1994). Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease. *NeuroReport* 6, 49-53.

Hornykiewicz, O. (1974). The mechanisms of action of l-dopa in Parkinson's disease. *Life Sci.* 15, 1249-1259.

Hou, J.-G. G., Lin, L.-F. H., and Mytilineou, C. (1996). Glial cell line-derived neurotrophic factor exerts neurotrophic effects on dopaminergic neurons in vitro and promotes their survival and regrowth after damage by 1-methyl-4-phenylpyridinium. *J. Neurochem.* 66, 74-82.

Hudson, J., Granholm, A.-C., Gerhardt, G. A., Henry, M. A., Hoffman, A., Biddle, P., Leela, N. S., Mackerlova, L., Lile, J. D., Collins, F., and Hoffer, B. J. (1995). Glial cell-line derived neurotrophic factor augments midbrain dopaminergic circuits in vivo. *Brain Res. Bull.* 36, 425-432.

Huffaker, T. K., Boss, B. D., Morgan, A. S., Neff, N. T., Strecker, R. E., Spence, M. S., and Miao, R. (1989). Xenografting of fetal pig ventral mesencephalon corrects motor asymmetry in the rat model of Parkinson's disease. *Brain Res.* 77, 329-336.

Hyman, C., Hofer, M., Barde, Y.-A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P., and Lindsay, R. M. (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350, 230-232.

Hyman, C., Juhasz, M., Jackson, C., Wright, P., Ip, N. Y., and Lindsay, R. M. (1994). Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *Neuroscience* 14, 335-347.

Hynes, M. A., Poulsen, K., Armanini, M., Berkemeier, L., Phillips, H., and Rosenthal, A. (1994). Neurotrophin-4/5 is a survival factor for embryonic midbrain dopaminergic neurons in enriched cultures. *J. Neurosci. Res.* 37, 144-154.

Iacopino, A. M., and Christakos, S. (1990). Specific reduction of calcium-binding protein (280 kilodalton calbindin-D) gene expression in aging and neurodegenerative diseases. *Proc. Natl. Acad. Sci., USA* 87, 4078-4082.

Inoue, H., Kohsaka, S., Yoshida, K., Ohtani, M., Toya, S., and Tsukada, Y. (1985). Cyclosporin A enhances the survivability of mouse cerebral cortex grafted into the third ventricle of rat brain. *Neurosci. Lett.* 54, 85-90.

Ip, N. Y., Ibanez, C. F., Nye, S. H., McClain, J., Jones, P. F., Gies, D. R., Belluscio, L., LeBeau, M. M., Espinosa, R., Squinto, S. P., Persson, H., and Yancopoulos, G. D. (1992). Mammalian neurotrophin-4: Structure, chromosomal localization, tissue distribution, and receptor specificity. *Proc. Natl. Acad. Sci., USA* 89, 3060-3064.

Isacson, O. (1993). On neuronal health. *Trends Neurosci.* 16, 306-308.

Isacson, O. (1995). Gene delivery in parkinson rat model. *Science* 269, 856-857.

Isacson, O., Brundin, P., Gage, F. H., and Björklund, A. (1985). Neural grafting in a rat model of Huntington's disease: progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. *Neuroscience* 16, 799-817.

Isacson, O., Deacon, T. W., Pakzaban, P., Galpern, W. R., Dinsmore, J., and Burns, L. H. (1995). Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibers. *Nature Medicine* 1, 1189-1194.

Ischiropoulos, H., Zhu, L., Chen, J., Tsai, M., Martin, J. C., Smith, C. D., and Beckman, J. S. (1992). Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch. Biochem. Biophys.* 298, 431-437.

Jackson, G. R., Apffel, L., Werrbach-Perez, K., and Perez-Polo, J. R. (1990). Role of nerve growth factor in oxidant-antioxidant balance and neuronal injury. I. Stimulation of hydrogen peroxide resistance. *J. Neurosci. Res.* 25, 360-368.

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., and Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. Natl. Acad. Sci. USA* 82, 2173-2177.

Javitch, J. A., Uhl, G. R., and Snyder, S. H. (1984). Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Characterization and localization of receptor binding sites in rat and human brain. *Proc. Natl. Acad. Sci. USA* 81, 4591-4595.

Jellinger, K. (1986). Overview of morphological changes in Parkinson's disease. In *Advances in Neurology*, M. D. Yahr and K. J. Bergmann, eds. (New York: Raven Press), pp. 1-18.

Jelsma, T. N., and Aguayo, A. J. (1994). Trophic factors. *Curr. Opin. Neurobiol.* 4, 717-725.

Jenkins, B. G., Koroshetz, W. J., Beal, M. F., and Rosen, B. R. (1993). Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized ¹H NMR spectroscopy. *Neurology* 43, 2689-2695.

Johnson, E. M. J., Koike, T., and Franklin, J. (1992). A "calcium set-point hypothesis" of neuronal dependence on neurotrophic factor. *Exp. Neurol.* 115, 163-166.

Jones, K. R., and Reichardt, L. F. (1990). Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proc. Natl. Acad. Sci., USA* 87, 8060-8064.

Jonsson, G. (1980). Chemical neurotoxins as denervation tools in neurobiology.

Ann. Rev. Neurosci. 3, 169-187.

Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V., and Parada, L. F.

(1991a). The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science* 252, 554-558.

Kaplan, D. R., Martin-Zanca, D., and Parada, L. F. (1991b). Tyrosine

phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. *Nature* 350, 158-160.

Kaplitt, M. J., Leone, P., Samulski, R. J., Xiao, X., Pfaff, D. W., O'Malley, K. L., and

During, M. J. (1994). Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nature Genetics* 8, 148-154.

Kawaja, M. D., Rosenberg, M. B., Yoshida, K., and Gage, F. H. (1992). Somatic

gene transfer of nerve growth factor promotes the survival of axotomized septal neurons and the regeneration of their axons in adult rats. *J. Neurosci.* 12, 2849-2864.

Kearns, C. M., and Gash, D. M. (1995). GDNF protects nigral dopamine neurons against 6-hydroxydopamine in vivo. *Brain Res.* 672, 104-111.

Kennedy, T. E., Serafini, T., de la Torre, J. R., and Tessier-Lavigne, M. (1994). Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 78, 425-435.

Kish, S. J., Morito, C., and Hornykiewicz, O. (1985). Glutathione peroxidase activity in Parkinson's disease brain. *Neurosci. Lett.* 58, 343-346.

Kish, S. J., Shannak, K., and Hornykiewicz, O. (1988). Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. *New Engl. J. Med.* 318, 876-880.

Klein, R., Conway, D., Parada, L., and Barbacid, M. (1990). The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. *Cell* 61, 647-656.

Klein, R., Jing, S., Nanduri, V., O'Rourke, E., and Barbacid, M. (1991a). The trk proto-oncogene encodes a receptor for nerve growth factor. *Cell* 65, 189-197.

Klein, R., Lamballe, F., Bryant, S., and Barbacid, M. (1992). The trkB tyrosine protein kinase is a receptor for neurotrophin-4. *Neuron* 8, 947-956.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., and Barbacid, M. (1991b). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell* 66, 395-403.

Knusel, B., Beck, K. D., Winslow, J. W., Rosenthal, A., Burton, L. E., Widmer, H. R., Nikolics, K., and Hefti, F. (1992). Brain-derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degenerative changes after axotomy in the adult rat brain. *J. Neurosci.* 12, 4391-4402.

Knusel, B., Michel, P. P., Schwaber, J. S., and Hefti, F. (1990). Selective and non-selective stimulation of central cholinergic and dopaminergic development in vitro by nerve growth factor, basic fibroblast growth factor, epidermal growth factor, insulin and the insulin-like growth factors I and II. *J. Neurosci.* 10, 558-570.

Knusel, B., Winslow, J. W., Rosenthal, A., Burton, L. E., Seid, D. P., Nikolics, K., and Hefti, F. (1991). Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin-3. *Proc. Natl. Acad. Sci. USA* 88, 961-965.

Kokaia, M., Bengzon, J., Metsis, M., Kokaia, M., Persson, H., and Lindvall, O. (1993). Coexpression of neurotrophins and their receptors in neurons of the central nervous system. *Proc. Natl. Acad. Sci., USA* 90, 6711-6715.

Koller, W. C., and Hubble, J. P. (1990). Levodopa therapy in Parkinson's disease. *Neurology* 40S, 40-47.

Kordower, J. H., Charles, V., Bayer, R., Bartus, R. T., Putney, S., Walus, L. R., and Friden, P. M. (1994a). Intravenous administration of a transferrin receptor antibody-nerve growth factor conjugate prevents the degeneration of cholinergic neurons in a model of Huntington's disease. *Proc. Natl. Acad. Sci., USA* 91, 9077-9080.

Kordower, J. H., Freeman, T. B., Snow, B. J., Vingerhoets, F. J. G., Mufson, E. J., Sanberg, P. R., Hauser, R. A., Smith, D. A., Nauert, G. M., Perl, D. P., and Olanow, C. W. (1995). Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N. Engl. J. Med.* 332, 1118-1124.

Kordower, J. H., Mufson, E. J., Granholm, A.-C., Hoffer, B., and Friden, P. M. (1994b). Delivery of trophic factors to the primate brain. *Exp. Neurol.* 124, 21-30.

Korsching, S. (1993). The neurotrophic factor concept: a reexamination. *J. Neurosci.* 13, 2739-2748.

Kowall, N. W., Ferrante, R. J., and Martin, J. B. (1987). Patterns of cell loss in Huntington's disease. *Trends Neurosci.* 10, 24-29.

Kriegstein, K., and Unsicker, K. (1994). Transforming growth factor- β promotes survival of midbrain dopaminergic neurons and protects them against N-methyl-4-phenylpyridinium ion toxicity. *Neuroscience* 63, 1189-1196.

Kromer, L. F. (1987). Nerve growth factor treatment after brain injury prevents neuronal death. *Science* 235, 214-216.

Laitinen, L. (1995). Pallidotomy for Parkinson's disease. *Neurosurgery Clinics of North America* 6, 105-112.

Laitinen, L. V., Bergenheim, A. T., and Hariz, M. I. (1992). Leksell's posteroventral pallidotomy in the treatment of Parkinson's disease. *J. Neurosurg.*

Lamballe, F., Klein, R., and Barbacid, M. (1991). trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell* 66, 967-979.

Lampson, L. A. (1987). Molecular bases of the immune response to neural antigens. *Trends Neurosci.* 10, 211-216.

Lams, B. E., Isacson, O., and Sofroniew, M. V. (1988). Loss of transmitter-associated enzyme staining following axotomy does not indicate death of brainstem cholinergic neurons. *Brain Res.* 475, 401-406.

Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. (1983). Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219, 979-980.

Langston, J. W., Irwin, I., Langston, E. B., and Forno, L. S. (1984). Pargyline prevents MPTP-induced parkinsonism in primates. *Science* 225, 1480-2.

Lapchak, P. A., Beck, K. D., Araujo, D. M., Irwin, I., Langston, J. W., and Hefti, F. (1993). Chronic intranigral administration of brain-derived neurotrophic factor produces striatal dopaminergic hypofunction in unlesioned adult rats and fails to attenuate the decline of striatal dopaminergic function following medial forebrain bundle transection. *Neuroscience* 53, 639-650.

Lawrence, J. M., Morris, R. J., Wilson, D. J., and Raisman, G. (1990). Mechanisms of allograft rejection in the rat brain. *Neuroscience* 37, 431-462.

Le Gal La Salle, G., Robert, J. J., Berrard, S., Ridoux, V., Stratford-Perricaudet, L. D., Perricaudet, M., and Mallet, J. (1993). An adenovirus vector for gene transfer into neurons and glia in the brain. *Science* 259, 988-990.

Lee, T., Seeman, P., Rajput, A., Farley, I. J., and Hornykiewicz, O. (1978). Receptor basis for dopaminergic supersensitivity in Parkinson's disease. *Nature* 273, 59-61.

Lehrach, H., Diamond, D., Wozney, J. M., and Boedtker, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* 16, 4743-4751.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., and Barde, Y.-A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 341, 149-152.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science* 237, 1154-1162.

Levitt, P., Pintar, J. E., and Breakefield, X. O. (1982). Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc. Natl. Acad. Sci. USA* 79, 6385-6389.

Levivier, M., Przedborski, S., Bencsics, C., and Kang, U. J. (1995). Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J. Neurosci.* 15, 7810-7820.

Lin, L.-F. H., Doherty, D. H., Lile, J. D., Bektesh, S., and Collins, F. (1993). GDNF: a glial cell-line derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260, 1130-1132.

Lin, L.-F. H., Mismer, D., Lile, J. D., Armes, L. G., Butler III, E. T., Vannice, J. L., and Collins, F. (1989). Purification, cloning, and expression of ciliary neurotrophic factor (CNTF). *Science* 246, 1023-1025.

Lindsay, R. M., Altar, C. A., Cedarbaum, J. M., Hyman, C., and Wiegand, S. J. (1993). The therapeutic potential of neurotrophic factors in the treatment of Parkinson's disease. *Exp Neurol* 124, 103-18.

Lindvall, O. (1991). Prospects of transplantation in human neurodegenerative diseases. *Trends Neurosci.* 8, 376-384.

Lindvall, O. (1995). Neural transplantation. *Cell Transpl.* 4, 393-400.

Lindvall, O., Backlund, E.-O., Farde, L., Sedvall, G., Freedman, R., Hoffer, B., Nobin, A., Seiger, Å., and Olson, L. (1987). Transplantation in Parkinson's disease: two cases of adrenal medullary grafts to the putamen. *Ann. Neurol.* 22, 457-468.

Lindvall, O., Brundin, P., Widner, H., Rehnström, S., Gustavii, B., Frackowiak, R., Leenders, K. L., Sawle, G., Rothwell, J. C., Marsden, C. D., and Björklund, A. (1990a). Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 247, 574-7.

Lindvall, O., Rehnström, S., Brundin, P., Gustavii, B., Åstedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K. L., Rothwell, J. C., Frackowiak, R., Marsden, C. D., Johnels, B., Steg, G., Freedman, R., Hoffer, B. J., Seiger, Å., Bygdeman, M., and Olson, L. (1990b). Neural transplantation in Parkinson's disease: the Swedish experience. *Prog. Brain Res.* 82, 729-734.

Lindvall, O., Rehnström, S., Brundin, P., Gustavii, B., Åstedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K. L., Rothwell, J. C., Frackowiak, R., Marsden, C. D., Johnels, B., Steg, G., Freedman, R., Hoffer, B. J., Seiger, Å., Bygdeman, M., Strömberg, I., and Olson, L. (1989). Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. *Arch. Neurol.* 46, 615-631.

Lindvall, O., Sawle, G., Widner, H., Rothwell, J. C., Björklund, A., Brooks, D., Brundin, P., Frackowiak, R., Marsden, C. D., Odin, P., and Rehnström, S. (1994). Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann. Neurol.* 35, 172-180.

Lindvall, O., Widner, H., Rehnström, S., Brundin, P., Odin, P., Gustavii, B., Frackowiak, R., Leenders, K. L., Sawle, G., Rothwell, J. C., Björklund, A., and Marsden, C. D. (1992). Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. *Ann. Neurol.* 31, 155-65.

Ljungberg, T., and Ungerstedt, U. (1976). Sensory inattention produced by 6-hydroxydopamine-induced degeneration of ascending dopamine neurons in the brain. *Exp. Neurol.* 53, 585-600.

Low, W. C., Lewis, P. R., and Bunch, S. T. (1983). Embryonic neural transplants across a major histocompatibility barrier: survival and specificity of innervation. *Brain Res.* 262, 328-333.

MacKenzie, G. M., Jenner, P., and Marsden, C. D. (1995). Effect of 7-nitro indazole on quinolinic acid-induced toxicity in the rat. *Neuroreport* 6, 1789-1794.

Madrazo, I., Drucker-Colin, R., Diaz, V., Martinez-Mata, J., Torres, C., and Becerril, J. J. (1987). Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N. Engl. J. Med.* 316, 831-834.

Mahalik, T. J., and Clayton, G. H. (1991). Specific outgrowth from neurons of ventral mesencephalic grafts to the catecholamine-depleted striatum of adult hosts. *Exp. Neurol.* 113, 18-27.

Mahalik, T. J., Finger, T. E., Strömberg, I., and Olson, L. (1985). Substantia nigra transplants into denervated striatum of the rat: Ultrastructure of graft and host interconnections. *J. Comp. Neurol.* 240, 60-70.

Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., and Yancopoulos, G. D. (1990). Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* 247, 1446-1451.

Manier, M., Abrous, D. N., Feuerstein, C., LeMoal, M., and Herman, J. P. (1991). Increase of striatal methionin enkephalin content following lesion of the nigrostriatal dopaminergic pathway in adult rats and reversal following the implantation of embryonic dopaminergic neurons: a quantitative immunohistochemical analysis. *Neuroscience* 42, 427-439.

Mann, V. M., Cooper, J. M., Javoy-Agid, F., Agid, Y., Jenner, P., and Schapira, A. H. V. (1990). Mitochondrial function and parental sex effect in Huntington's disease. *Lancet* 336, 749.

Maraganore, D. M., Harding, A. E., and Marsden, C. D. (1991). A clinical and genetic study of familial Parkinson's disease. *Movement Disorders* 6, 205-211.

Markopoulou, K., Wszolek, Z. K., and Pfeiffer, R. F. (1995). A Greek-American kindred with autosomal dominant, levodopa-responsive parkinsonism and anticipation. *Ann. Neurol.* 38, 373-378.

Marsden, C. D. (1982a). Basal ganglia disease. *Lancet*, 1141-1147.

Marsden, C. D. (1982b). The mysterious motor function of the basal ganglia: the Robert Wartenberg lecture. *Neurology* 32, 514-539.

Marsden, C. D. (1987). Parkinson's disease in twins. *J. Neurol. Neurosurg. Psych.* 30, 105-106.

Marsden, C. D., and Parkes, J. D. (1976). "On-off" effects in patients with Parkinson's disease in chronic levodopa therapy. *Lancet*, 292-296.

Marsden, C. D., and Parkes, J. D. (1977). Success and problems of long-term levodopa therapy in Parkinson's disease. *Lancet* 1, 345-349.

Marshall, J. F., and Ungerstedt, U. (1977). Supersensitivity to apomorphine following destruction of the ascending dopamine neurons: Quantification using the rotational model. *Eur. J. Pharmacol.* 41, 361-367.

Martin, J. B., and Gusella, J. F. (1986). Huntington's disease pathogenesis and management. *N. Engl. J. Med.* 315, 1268-1276.

Martin-Iverson, M. T., Todd, K. G., and Altar, C. A. (1994). Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine. *J. Neurosci.* 14, 1262-1270.

Martinez-Serrano, A., Lundberg, C., Horellou, P., Fischer, W., Bentlage, C., Campbell, K., McKay, R. D. G., Mallet, J., and Björklund, A. (1995). CNS-derived neural progenitor cells for gene transfer of nerve growth factor to the adult brain: complete rescue of axotomized cholinergic neurons after transplantation into the septum. *J. Neurosci.* 15, 5668-5680.

Marttila, R. J., Kaprio, J., Koskenvuo, M., and Rinne, U. K. (1988). Parkinson's disease in a nationwide twin cohort. *Neurology* 38, 1217-1219.

Marttila, R. J., and Rinne, U. K. (1980). Smoking and Parkinson's disease. *Acta Neurol. Scand.* 62, 322-325.

Marttila, R. J., and Rinne, U. K. (1981). Epidemiology of Parkinson's disease - An overview. *Neural Trans.* 51, 135-148.

Mason, D. W., Charlton, H. M., Jones, A. J., Lavy, C. B. D., Puklavec, M., and Simmonds, S. J. (1986). The fate of allogeneic and xenogeneic neuronal tissue transplanted into the third ventricle of rodents. *Neuroscience* 19, 685-694.

Massague, J. (1992). Receptors for the TGF-beta family. *Cell* 69, 1067-1070.

Mattson, M. P., Cheng, B., and Smith-Swintosky, V. L. (1993). Mechanisms of neurotrophic factor protection against calcium- and free radical-mediated excitotoxic injury: implications for treating neurodegenerative disorders. *Exp. Neurol.* 124, 89-95.

Mattson, M. P., Lovell, M. A., Furukawa, K., and Markesbery, W. R. (1995). Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular Ca^{2+} concentration, and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons. *J. Neurochem.* 65, 1740-1751.

Mayer, E., Dunnett, S. B., Pellitteri, R., and Fawcett, J. W. (1993a). Basic fibroblast growth factor promotes the survival of embryonic ventral mesencephalic dopaminergic neurons-I. Effects in vitro. *Neuroscience* 56, 379-388.

Mayer, E., Fawcett, J. W., and Dunnett, S. B. (1993b). Basic fibroblast growth factor promotes the survival of embryonic ventral mesencephalon dopaminergic neurons-II. Effects on nigral transplants in vivo. *Neuroscience* 56, 389-398.

McGeer, P. L., McGeer, E. G., and Suzuki, J. S. (1977). Aging and extrapyramidal function. *Arch. Neurol.* 34, 33-35.

Meakin, S. O., and Shooter, E. M. (1992). The nerve growth factor family of receptors. *Trends Neurosci.* 15, 323-330.

Melamed, E. (1986). Initiation of levodopa therapy in parkinsonian patients should be delayed until the advanced stages of the disease. *Arch. Neurol.* 43, 402-405.

Mendez, I. M., Naus, C. C. G., Elisevich, K., and Flumerfelt, B. A. (1993). Normalization of striatal proenkephalin and preprotachykinin mRNA expression by fetal substantia nigra grafts. *Exp. Neurol.* 119, 1-10.

Merlio, J.-P., Ernfors, P., Jaber, M., and Persson, H. (1992). Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience* 51, 513-532.

Michel, P. P., and Hefti, F. (1990). Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. *J. Neurosci. Res.* 26, 428-435.

Middlemas, D. S., Lindberg, R. A., and Hunter, T. (1991). trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol. Cell. Biol.* 11, 143-153.

Miller, R. G., Petajan, J. H., Bryan, W. W., Armon, C., Barohn, R. J., Goodpasture, J. C., Hoagland, R. J., Parry, G. J., Ross, M. A., Stromatt, S. C., and The rhCNTF ALS Study Group (1996). A Placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. *Ann. Neurol.* 39, 256-260.

Miranda, R. C., Sohrabji, F., and Toran-Allerand, C. D. (1993). Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. *Proc. Natl. Acad. Sci., USA* 90, 6439-6443.

Mitsumoto, H., Ikeda, K., Klinkosz, B., Cedarbaum, J. M., Wong, V., and Lindsay, R. M. (1994). Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. *Science* 265, 1107-1110.

Montoya, C. P., Astell, S., and Dunnett, S. B. (1990). Effects of nigral and striatal grafts on skilled forelimb use in the rat. *Prog. Brain Res.* 82, 459-466.

Morgan, D. G. (1989). Considerations in the treatment of neurological disorders with trophic factors. *Neurobiol. Aging* 10, 547-549.

Morse, J. K., Wiegand, S. J., Anderson, K., You, Y., Cai, N., Carnahan, J., Miller, J., DiStefano, P. S., Altar, C. A., Lindsay, R. M., and Alderson, R. F. (1993). Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. *J. Neurosci.* 13, 4146-4156.

Moukhles, H., Nieoullon, A., and Daszuta, A. (1992). Early and widespread normalization of dopamine-neuropeptide Y interactions in the rat striatum after transplantation of fetal mesencephalon cells. *Neuroscience* 47, 781-792.

Mouradian, M. M., Heuser, I. J. E., Baronti, F., and Chase, T. N. (1989). Modification of central dopaminergic mechanisms by continuous levodopa therapy for advanced Parkinson's disease. *Ann. Neurol.* 27, 18-22.

Mufson, E. J., Kroin, J. S., Sobreviela, T., Burke, M. A., Kordower, J. H., Penn, R. D., and Miller, J. A. (1994). Intrastratial infusions of brain-derived neurotrophic factor: retrograde transport and colocalization with dopamine containing substantia nigra neurons in rat. *Exp. Neurol.* 129, 15-26.

Nakao, N., Frodl, E. M., Duan, W.-M., Widner, H., and Brundin, P. (1994). Lazaroids improve the survival of grafted rat embryonic dopamine neurons. *Proc. Natl. Acad. Sci., USA* 91, 12408-12412.

Nakao, N., Frodl, E. M., Widner, H., Carlson, E., Eggerding, F. A., Epstein, C. J., and Brundin, P. (1995). Overexpressing Cu/Zn superoxide dismutase enhances survival of transplanted neurons in a rat model of Parkinson's disease. *Nature Medicine* 1, 226-231.

Neumann, H., Cavalié, A., Jenne, D., and Wekerle, H. (1995). Induction of MHC class I genes in neurons. *Science* 269, 549-552.

Nicholas, M. K., Chenelle, A. G., Brown, M. M., Stefansson, K., and Arnason, B. G. W. (1990). Prevention of neural allograft rejection in the mouse following in vivo depletion of L3T4+ but not LYT-2+ T-lymphocytes. *Prog. Brain Res.* 82, 161-167.

Nicklas, W. J., Vyas, I., and Heikkila, R. E. (1985). Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci* 36, 2503-8.

Nicklas, W. J., Youngster, S. K., Kindt, M. V., and Heikkila, R. E. (1987). MPTP, MPP+ and mitochondrial function. *Life Sci* 40, 721-9.

Nikkhah, G., Cunningham, M. G., Jödicke, A., Knappe, U., and Björklund, A. (1994). Improved graft survival and striatal reinnervation by microtransplantation of fetal nigral cell suspensions in the rat Parkinson model. *Brain Res.* 633, 133-143.

Nikkhah, G., Duan, W.-M., Knappe, U., Jödicke, A., and Björklund, A. (1993a). Restoration of complex sensorimotor behavior and skilled forelimb use by a modified nigral suspension transplantation approach in the rat parkinson model. *Neuroscience* 56, 33-43.

Nikkhah, G., Eberhard, J., Olsson, M., and Björklund, A. (1995). Preservation of fetal ventral mesencephalic cells by cool storage: in-vitro viability and TH-positive neuron survival after microtransplantation to the striatum. *Brain Res.* 687, 22-34.

Nikkhah, G., Odin, P., Smits, A., Tingstrom, A., Othberg, A., Brundin, P., Funa, K., and Lindvall, O. (1993b). Platelet-derived growth factor promotes survival of rat and human mesencephalic dopaminergic neurons in culture. *Exp. Brain Res.* 92, 516-523.

Nisticò, G., Ciriolo, M. R., Fiskin, K., Iannone, M., De Martino, A., and Rotilio, G. (1992). NGF restores decrease in catalase activity and increases superoxide dismutase and glutathione peroxidase activity in the brain of aged rats. *Free Radic. Biol. Med.* 12, 177-181.

Novelli, A., Reilly, J. A., Lysko, P. G., and Henneberry, R. C. (1988). Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res.* 451, 205-212.

Nyberg, P., Nordberg, A., Wester, P., and Winblad, B. (1983). Dopaminergic deficiency is more pronounced in putamen than in nucleus caudatus in Parkinson's disease. *Neurochem. Pathol.* 1, 193-202.

Oettinger, H. F., Rodrique-Way, A., Bousquet, J. J., and Edge, A. S. B. (1995). Porcine repeat element DNA: in situ detection of xenotransplanted cells. *Cell Transplantation* 4, 253-256.

Olanow, C. W. (1990). Oxidation reactions in Parkinson's disease. *Neurology* 40, 32-37.

Otto, D., and Unsicker, K. (1990). Basic FGF reverses chemical and morphological deficits in the nigrostriatal system of MPTP-treated mice. *J. Neurosci.* 10, 1912-1921.

Pakzaban, P., Deacon, T. W., Burns, L. H., Dinsmore, J., and Isacson, O. (1995). A novel mode of immunoprotection of neural xenotransplants: masking of donor major histocompatibility complex class I enhances transplant survival in the central nervous system. *Neuroscience* 65, 983-996.

Pakzaban, P., and Isacson, O. (1994). Neural xenotransplantation: reconstruction of neuronal circuitry across species barriers. *Neuroscience* 62, 989-1001.

- Palmer, T. D., Rosman, G. J., Osborne, W. R. A., and Miller, A. D. (1991). Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes. *Proc. Natl. Acad. Sci., USA* 88, 1330-1334.
- Parboosingh, J. S., Rousseau, M., Rogan, F., Amit, Z., Chertkow, H., Johnson, W. G., Manganaro, F., Schipper, H. N., Curran, T. J., Stoessl, J., and Rouleau, G. A. (1995). Absence of mutations in superoxide dismutase and catalase genes in patients with Parkinson's disease. *Arch. Neurol.* 52, 1160-1163.
- Park, T. H., and Mytilineou, C. (1992). Protection from 1-methyl-4-phenylpyridinium (MPP+) toxicity and stimulation of regrowth of MPP+-damaged dopaminergic fibers by treatment of mesencephalic cultures with EGF and basic FGF. *Brain Res.* 599, 83-97.
- Parker, W. D., Boyson, S. J., Luder, A. S., and Parks, J. K. (1990). Evidence for a defect in NADH:ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* 40, 1231-1234.
- Parker, W. D., Jr., Boyson, S. J., and Parks, J. K. (1989). Abnormalities of the electron transport chain in idiopathic Parkinson's disease. *Ann. Neurol.* 26, 719-723.
- Parkinson Study Group (1989). Effect of deprenyl on the progression of disability in early Parkinson's disease. *N. Engl. J. Med.* 321, 1364-1371.

Parkinson Study Group (1993). Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. *N. Engl. J. Med.* 328, 176-183.

Parkinson Study Group (1996a). Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP patients requiring levodopa. *Ann. Neurol.* 39, 37-45.

Parkinson Study Group (1996b). Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP subjects not requiring levodopa. *Ann. Neurol.* 39, 29-36.

Patino, P., Kriek, E. H., Hutt, C. J., Qi, J. X., and Freed, C. R. (1992). Short term corticosteroid treatment impairs survival of fetal dopamine cells transplanted to a rat model of Parkinson's disease. *Soc. Neurosci. Abstr.* 18, 59.

Payami, H., Larsen, K., Bernard, S., and Nutt, J. (1994). Increased risk of Parkinson's disease in parents and siblings of patients. *Ann. Neurol.* 36, 659-661.

Pedersen, E. B., Poulson, F. R., Zimmer, J., and Finsen, B. (1995). Prevention of mouse-rat xenograft rejection by a combination therapy of cyclosporin A, prednisolone and azathioprine. *Exp. Brain Res.* 106, 181-186.

Perlow, M. J., Freed, W. J., Hoffer, B. J., Seiger, A., Olson, L., and Wyatt, R. J. (1979). Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 204, 643-647.

Perry, T. L., Godin, D. V., and Hansen, S. (1982). Parkinson's disease: a disorder due to nigral glutathione deficiency? *Neurosci. Lett.* 33, 305-310.

Perry, T. L., Hansen, S., and Kloster, M. (1973). Huntington's chorea: deficiency of gamma-aminobutyric acid in brain. *N. Engl. J. Med.* 288, 337-342.

Peschanski, M., Defer, G., N'Guyen, J. P., Ricolfi, F., Monfort, J. C., Remy, P., Geny, C., Samson, Y., Hantraye, P., Jeny, R., Gaston, A., Kéravel, Y., Degos, J. D., and Cesaro, P. (1994). Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. *Brain* 117, 487-499.

Poulson, K. T., Armanini, M. P., Klein, R. D., Hynes, M. A., Phillips, H. S., and Rosenthal, A. (1994). TGF β 2 and TGF β 3 are potent survival factors for midbrain dopaminergic neurons. *Neuron* 13, 1245-1252.

Przedborski, S., Jackson-Lewis, V., Muthane, U., Jiang, H., Ferreira, M., Naini, A. B., and Fahn, S. (1993). Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. *Ann. Neurol.* 34, 715-723.

Purves, D. (1986). The trophic theory of neural connections. *Trends Neurosci.* 9, 486-489.

Rajput, A. H. (1992). Frequency and cause of Parkinson's disease. *Can. J. Neurol. Sci.* 19, 103-107.

Redmond, D. E., Naftolin, F., Collier, T. J., Leranth, C., Robbins, R. J., Sladek, C. D., Roth, R. H., and Sladek, J. R. (1988). Cryopreservation, culture, and transplantation of human fetal mesencephalic tissue into monkeys. *Science* 242, 768-771.

Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C., Penney, J. B., and Young, A. B. (1988). Differential loss of striatal projection neurons in Huntington's disease. *Proc. Natl. Acad. Sci., USA* 85, 5733-5737.

Richardson, P. M., Verge Issa, V. M. K., and Riopelle, R. J. (1986). Distribution of neuronal receptors for nerve growth factor in the rat. *J. Neurosci.* 6, 2312-2321.

Rioux, L., Gaudin, D. P., Gagnon, C., DiPaola, T., and Bédard, P. J. (1991). Decrease of behavioral and biochemical denervation supersensitivity of rat striatum by nigral transplants. *Neuroscience* 44, 75-83.

Robertson, H. A. (1992). Dopamine receptor interactions: some implications for the treatment of Parkinson's disease. *Trends Neurosci.* 15, 201-206.

Rodriguez-Tebar, A., Dechant, G., and Barde, Y.-A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* 4, 487-492.

Rosenberg, M. B., Friedmann, T., Robertson, R. C., Tuszynski, M., Wolff, J. A., Breakefield, X. O., and Gage, F. H. (1988). Grafting genetically modified cells to the damaged brain: restorative effects of NGF expression. *Science* 242, 1575-1578.

Rosenthal, A., Goeddel, D. V., Nguyen, T., Shih, A., Laramée, G. R., Nikolics, K., and Winslow, J. W. (1990). Primary structure and biological activity of a novel human neurotrophic factor. *Neuron* 4, 767-773.

Rothman, S. M. (1985). The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *J. Neurosci.* 5, 1483-1489.

Sabaté, O., Horellou, P., Vigne, E., Colin, P., Perricaudet, M., Buc-Caron, M.-H., and Mallet, J. (1995). Transplantation to the rat brain of human neural progenitors that were genetically modified using adenovirus. *Nature Genetics* 9, 256-260.

Sacher, G. A., and Staffeldt, E. F. (1974). Relation of gestation time to brain weight in placental mammals. *Amer. Natur.* 108, 593-616.

Sasahara, M., Fries, J. W., Raines, E. W., Gowan, A. M., Westrum, L. E., Frosch, M. P., Bonthron, D. T., Ross, R., and Collins, T. (1991). PDGF B-chain in neurons

of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* 64, 217-227.

Sauer, H., and Brundin, P. (1991). Effects of cool storage on survival and function of intrastriatal ventral mesencephalic grafts. *Restor. Neurol. Neurosci.* 2, 123-135.

Sauer, H., Fischer, W., Nikkhah, G., Wiegand, S. J., Brundin, P., Lindsay, R. M., and Bjorklund, A. (1993). Brain-derived neurotrophic factor enhances function rather than survival of intrastriatal dopamine cell-rich grafts. *Brain Res.* 626, 37-44.

Sauer, H., Frodl, E. M., Kupsch, A., ten Bruggencate, G., and Oertel, W. H. (1992). Cryopreservation, survival and function of intrastriatal fetal mesencephalic grafts in a rat model of Parkinson's disease. *Exp. Brain Res.* 90, 54-62.

Sauer, H., Rosenblad, C., and Björklund, A. (1995a). Glial cell line-derived neurotrophic factor but not transforming growth factor β 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc. Natl. Acad. Sci., USA* 92, 8935-8939.

Sauer, H., Wong, V., and Björklund, A. (1995b). Brain-derived neurotrophic factor and neurotrophin-4/5 modify neurotransmitter-related gene expression in the 6-hydroxydopamine-lesioned rat striatum. *Neuroscience* 65, 927-933.

- Sawle, G. V., Bloomfield, P. M., Bjorklund, A., Brooks, D. J., Brundin, P., Leenders, K. L., Lindvall, O., Marsden, C. D., Rehnström, S., Widner, H., and Frackowiak, R. S. J. (1992). Transplantation of fetal dopamine neurons in Parkinson's disease: PET [18F]6-L-fluorodopa studies in two patients with putaminal implants. *Ann Neurol* 31, 166-73.
- Schaar, D. G., Sieber, B.-A., Dreyfus, C. F., and Black, I. B. (1993). Regional and cell-specific expression of GDNF in rat brain. *Exp. Neurol.* 124, 368-371.
- Schapira, A. H. V., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., and Marsden, C. D. (1990). Mitochondrial complex 1 deficiency in parkinson's disease. *J. Neurochem.* 54, 823-827.
- Schapira, A. H. V., Cooper, J. M., Dexter, D., Jenner, P., Clark, J. B., and Marsden, C. D. (1989). Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* i, 1269.
- Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F., and Agid, Y. (1989). Striatal dopamine deficiency in Parkinson's disease: role of aging. *Ann. Neurol.* 26, 551-557.
- Schmidt, R. H., Björklund, A., and Stenevi, U. (1981). Intracerebral grafting of dissociated CNS tissue suspensions: a new approach for neuronal transplantation to deep brain sites. *Brain Res.* 218, 347-356.

Schmidt, R. H., Björklund, A., Stenevi, U., Dunnett, S. B., and Gage, F. H. (1983). Intracerebral grafting of neuronal cell suspensions III. Activity of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. *Acta Physiol. Scand. Suppl.* 522, 19-28.

Schmidt, R. H., Ingvar, M., Lindvall, O., Stenevi, U., and Björklund, A. (1982). Functional activity of substantia nigra grafts reinnervating the striatum: neurotransmitter metabolism and [^{14}C]2-deoxy-D-glucose autoradiography. *J. Neurochem.* 38, 737-48.

Schulz, J. B., Matthews, R. T., Jenkins, B. G., Ferrante, R. J., Siwek, D., Henshaw, D. R., Cipolloni, P. B., Mecocci, P., Kowall, N. W., Rosen, B. R., and Beal, M. F. (1995a). Blockade of neuronal nitric oxide synthase protects against excitotoxicity in vivo. *J. Neurosci.* 15, 8419-8429.

Schulz, J. B., Matthews, R. T., Muqit, M. M. K., Browne, S. E., and Beal, M. F. (1995b). Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J. Neurochem.* 64, 936-939.

Schulzer, M., Mak, E., and Calne, D. B. (1992). The antiparkinson efficacy of deprenyl derives from transient improvement that is likely to symptomatic. *Ann. Neurol.* 32, 795-798.

Schumacher, J. M., Short, M. P., Hyman, B. T., Breakefield, X. O., and Isacson, O. (1991). Intracerebral implantation of nerve growth factor-producing fibroblasts protects striatum against neurotoxic levels of excitatory amino acids. *Neuroscience* 45, 561-570.

Sendtner, M., Gnahn, H., Wakade, A., and Thoenen, H. (1988). Is activation of the Na⁺K⁺ pump necessary for NGF-mediated neuronal survival? *J. Neurosci.* 8, 458-462.

Sendtner, M., Holtmann, B., Kolbeck, R., Thoenen, H., and Barde, Y.-A. (1992a). Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* 360, 757-759.

Sendtner, M., Schmalbruch, H., Stockli, K. A., Carroll, P., Kreutzberg, G. W., and Thoenen, H. (1992b). Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuropathy. *Nature* 358, 502-504.

Serafini, T., Kennedy, T. E., Galko, M. J., Mirzayan, C., Jessell, T. M., and Tessier-Lavigne, M. (1994). The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. *Cell* 78, 409-424.

Seroogy, K. B., Lundgren, K. H., Tran, T. M. D., Guthrie, K. M., Isackson, P. J., and Gall, C. M. (1994). Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs. *J. Comp. Neurol.* 342, 321-334.

Shelton, D. L., and Reichardt, L. F. (1986). Studies on the expression of the β nerve growth factor (NGF) gene in the central nervous system: level and regional distribution of NGF mRNA suggest that NGF functions as a trophic factor for several distinct populations of neurons. *Proc. Natl. Acad. Sci., USA* 83, 2714-2718.

Shen, R.-Y., Altar, C. A., and Chiodo, L. A. (1994). Brain-derived neurotrophic factor increase the electrical activity of pars compacta dopamine neurons in vivo. *Proc. Natl. Acad. Sci., USA* 91, 8920-8924.

Shoffner, J. M., Watts, R. L., Juncos, J. L., Torroni, A., and Wallace, D. C. (1991). Mitochondrial oxidative phosphorylation defects in Parkinson's disease. *Ann. Neurol.* 30, 332-339.

Sian, J., Dexter, D. T., Lees, A. J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., and Marsden, C. D. (1994a). Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann. Neurol.* 36, 348-355.

Sian, J., Dexter, D. T., Lees, A. J., Daniel, S., Jenner, P., and Marsden, C. D. (1994b). Glutathione-related enzymes in brain in Parkinson's disease. *Ann. Neurol.* 36, 356-361.

Simpson, J. R., and Isacson, O. (1993). Mitochondrial impairment reduces the threshold for in vivo NMDA-mediated neuronal death in the striatum. *Exp. Neurol.* 121, 57-64.

Sloan, D. J., Barker, B. J., Puklavec, M., and Charlton, H. M. (1990). The effect of site of transplantation and histocompatibility differences on the survival of neural tissue transplanted to the CNS of defined inbred rat strains. *Prog. Brain Res.* 82, 141-152.

Sloan, D. J., Wood, M. J., and Charlton, H. M. (1991). The immune response to intracerebral neural grafts. *Trends Neurosci.* 14, 341-346.

Smits, A., Kato, M., Westermarck, B., Nistér, M., Heldin, C.-H., and Funa, K. (1991). Neurotrophic activity of platelet-derived growth factor (PDGF): rat neuronal cells possess functional PDGF β -type receptors and respond to PDGF. *Proc. Natl. Acad. Sci., USA* 88, 8159-8163.

Snow, M. H. L., and Tam, P. P. L. (1980). Timing in embryological development. *Nature* 286, 107.

Sofroniew, M. V., Galletly, N. P., Isacson, O., and Svendsen, C. N. (1990). Survival of adult basal forebrain cholinergic neurons after loss of target neurons. *Science* 247, 338-342.

Sofroniew, M. V., and Isacson, O. (1988). Distribution of degeneration of cholinergic neurons in the septum following axotomy in different portions of the fimbria fornix: a correlation between degree of cell loss and proximity of neuronal somata to the lesion. *J. Chem. Neuroanat.* 1, 327-337.

Soppet, D., Escandon, E., Maragos, J., Middlemas, D. S., Reid, S. W., Blair, J., Burton, L. E., Stanton, B. R., Kaplan, D. R., Hunter, T., Nikolics, K., and Parada, L. F. (1991). The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell* 65, 895-903.

Spina, M. B., and Cohen, G. (1989). Dopamine turnover and glutathione oxidation: Implications for Parkinson's disease. *Proc. Natl. Acad. Sci., USA* 86, 1398-1400.

Spina, M. B., Squinto, S. P., Miller, J., Lindsay, R. M., and Hyman, C. (1992). Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J. Neurochem.* 59, 99-106.

Springer, J. E., Mu, X., Bergman, L. W., and Trojanowski, J. Q. (1994). Expression of GDNF mRNA in rat and human nervous tissue. *Exp. Neurol.* 127, 167-170.

Squinto, S. P., Stitt, T. N., Aldrich, T. H., Davis, S., Bianco, S. M., Radziejewski, C., Glass, D. J., Masiakowski, P., Furth, M. E., Valenzuela, D. M., DiStefano, P. S., and Yancopoulos, G. D. (1991). *trkB* encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell* 65, 885-893.

Srivastava, R., Brouillet, E., Beal, M. F., Storey, E., and Hyman, B. T. (1993). Blockade of 1-methyl-4-phenylpyridinium ion (MPP⁺) nigral toxicity in the rat by prior decortication or MK-801 treatment: a stereological estimate of neuronal loss. *Neurobiol Aging* 14, 295-301.

Steininger, T. L., Wainer, B. H., Klein, R., Barbacid, M., and Palfrey, H. C. (1993). High-affinity nerve growth factor receptor (Trk) immunoreactivity is localized in cholinergic neurons of the basal forebrain and striatum in the adult rat brain. *Brain Res.* 612, 330-335.

Stenevi, U., Björklund, A., and Svendgaard, N.-A. (1976). Transplantation of central and peripheral monoamine neurons to the adult rat brain: Techniques and conditions for survival. *Brain Res.* 114, 1-20.

Stöckli, K. A., Lillien, L. E., Näher-Noé, M., Breitfeld, G., Hughes, R. A., Raff, M. C., and Thoenen, H. (1991). Regional distribution, developmental changes, and cellular localization of CNTF-mRNA and protein in the rat brain. *J. Cell Biol.* 115, 447-459.

Stöckli, K. A., Lottspeich, F., Sendtner, M., Masiakowski, P., Carroll, P., Gotz, R., Lindholm, D., and Thoenen, H. (1989). Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. *Nature* 342, 920-923.

Storey, E., Hyman, B. T., Jenkins, B., Brouillet, E., Miller, J. M., Rosen, B. R., and Beal, M. F. (1992). 1-Methyl-4-phenylpyridinium produces excitotoxic lesions in rat striatum as a result of impairment of oxidative metabolism. *J Neurochem* 58, 1975-1978.

Strecker, R. E., Sharp, T., Brundin, P., Zetterström, T., Ungerstedt, U., and Björklund, A. (1987). Autoregulation of dopamine release and metabolism by intrastriatal nigral grafts as revealed by intracerebral dialysis. *Neuroscience* 22, 169-178.

Strömberg, I., Almqvist, P., Bygdeman, M., Finger, T. E., Gerhardt, G., Granholm, A.-C., Mahalik, T. J., Seiger, A., Olson, L., and Hoffer, B. (1989). Human fetal mesencephalic tissue grafted to the dopamine-denervated striatum of athymic rats: Light- and electron-microscopical histochemistry ins in vivo chronoamperometric studies. *J. Neurosci.* 9, 614-624.

Strömberg, I., Björklund, L., Johansson, M., Tomac, A., Collins, F., Olson, L., Hoffer, B., and Humpel, C. (1993). Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons *in vivo*. *Exp. Neurol.* 124, 401-412.

Strömberg, I., Bygdeman, M., Goldstein, M., Seiger, Å., and Olson, L. (1986).

Human fetal substantia nigra grafted to the dopamine-denervated striatum of immunosuppressed rats: evidence for functional reinnervation. *Neurosci. Lett.* 71, 271-276.

Suthanthiran, M., and Strom, T. B. (1994). Renal transplantation. *N. Engl. J. Med.* 331, 365-376.

Takayama, H., Ray, J., Raymon, H. K., Baird, A., Hogg, J., Fisher, L. J., and Gage, F. H. (1995). Basic fibroblast growth factor increases dopaminergic graft survival and function in a rat model of Parkinson's disease. *Nature Medicine* 1, 53-58.

Tanner, C. M. (1989). The role of environmental toxins in the etiology of Parkinson's disease. *Trends Neurosci.* 12, 49-54.

Tanner, C. M. (1992). Epidemiology of Parkinson's disease. *Neurologic Clinics* 10, 317-329.

Tasker, R. R. (1990). Thalamotomy. *Neurosurgery Clinics of North America* 1, 841-864.

Ter Horst, G. J., Knigge, M. F., and Van der Wal, A. (1992). Neurochemical lesioning in the rat brain with iontophoretic injection of the 1-methyl-4-phenylpyridinium ion (MPP+). *Neurosci. Lett.* 141, 203-207.

Tetrud, J. W., and Langston, J. W. (1989). The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science* 245, 519-522.

The Huntington's Disease Collaborative Research Group (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72, 971-983.

Timmusk, T., Belluardo, N., Metsis, M., and Persson, H. (1993). Widespread and developmentally regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. *Eur. J. Neurosci.* 5, 605-613.

Tomac, A., Lindqvist, E., Lin, L.-F. H., Ogren, S. O., Young, D., Hoffer, B. J., and Olson, L. (1995a). Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. *Nature* 373, 335-339.

Tomac, A., Widenfalk, J., Lin, L.-F. H., Kohno, T., Ebendal, T., Hoffer, B. J., and Olson, L. (1995b). Retrograde axonal transport of glial cell-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc. Natl. Acad. Sci., USA* 92, 8274-8278.

Tsoufas, P., Soppet, D., Escandon, E., Tessarollo, L., Mendoza-Ramirez, J.-L., Rosenthal, A., Nikolics, K., and Parada, L. F. (1993). The rat trkC locus encodes multiple neurogenic receptors that exhibit differential response to neurotrophin-3 in PC12 cells. *Neuron* 10, 975-990.

Ungerstedt, U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmacol.* 5, 107-110.

Ungerstedt, U. (1971a). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine pathway. *Acta Physiol. Scand.*, Suppl. 367, 95-122.

Ungerstedt, U. (1971b). Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand.*, Suppl 367, 69-93.

Ungerstedt, U. (1971c). Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behavior. *Acta Physiol. Scand.*, Suppl 367, 49-68.

Ungerstedt, U., and Arbuthnott, G. W. (1970). Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res.* 24, 485-493.

Unsicker, K., Flanders, K. C., Cissel, D. S., Lafyatis, R., and Sporn, M. B. (1991). Transforming growth factor beta isoforms in the adult rat central and peripheral nervous system. *Neuroscience* 44, 613-625.

Valenzuela, D. M., Maisonpierre, P. C., Glass, D. J., Rojas, E., Nunez, L., Kong, Y., Gies, D. R., Stitt, T. N., Ip, N. Y., and Yancopoulos, G. D. (1993). Alternative forms of rat TrkC with different functional capabilities. *Neuron* 10, 963-974.

Varon, S., and Skaper, S. D. (1983). The Na⁺, K⁺ pump may mediate the control of nerve cells by nerve growth factor. *Trends Biol. Sci.*, 22-25.

Vejsada, R., Sagot, Y., and Kato, A. C. (1994). BDNF-mediated rescue of axotomized motor neurones decreases with increasing dose. *Neuroreport* 5, 1889-1892.

Venero, J. L., Beck, K. D., and Hefti, F. (1994). Intrastriatal infusion of nerve growth factor after quinolinic acid prevents reduction of cellular expression of choline acetyltransferase messenger RNA and trkA messenger RNA, but not glutamate decarboxylase messenger RNA. *Neuroscience* 61, 257-268.

Vierregge, P., Schiffke, K. A., Friedrich, H. J., Müller, B., and Ludin, H. P. (1992). Parkinson's disease in twins. *Neurology* 42, 1453-1461.

Wallace, D. C. (1992). Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 256, 628-632.

Wanaka, A., Johnson, E. M., and Milbrandt, J. (1990). Localization of FGF receptor mRNA in the adult rat central nervous system by in situ hybridization. *Neuron* 5, 267-281.

Ward, C. D., Duvoisin, R. C., Ince, S. E., Nutt, J. D., Eldridge, R., and Calne, D. B. (1983). Parkinson's disease in 65 pairs of twins and in a set of quadruplets.

Neurology 33, 815-824.

Waters, C. H., and Miller, C. A. (1994). Autosomal dominant lewy body parkinsonism in a four-generation family. *Ann. Neurol.* 35, 59-64.

Wekerle, H., Linington, C., Lassmann, H., and Metermann, R. (1986). Cellular immune reactivity within the CNS. *Trends Neurosci.* 9, 271-277.

Wetmore, C., Ernfors, P., Persson, H., and Olson, L. (1990). Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by in situ hybridization. *Exp. Neurol.* 109, 141-152.

Whishaw, I. Q., O'Connor, W. T., and Dunnett, S. B. (1986). The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in rat. *Brain* 109, 805-843.

Whittemore, S. R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, A., Strömberg, I., and Persson, H. (1986). Developmental and regional expression of β nerve growth factor messenger RNA and protein in the rat central nervous system. *Proc. Natl. Acad. Sci., USA* 83, 817-821.

Wictorin, K., Brundin, P., Sauer, H., Lindvall, O., and Björklund, A. (1992). Long distance directed axonal growth from human dopaminergic mesencephalic neuroblasts implanted along the nigrostriatal pathway in 6-hydroxydopamine lesioned adult rats. *J. Comp. Neurol.* 323, 475-494.

Widner, H. (1993). Immunologic aspects of intracerebral CNS tissue transplantation. In *Restoration of brain function by tissue transplantation*, O. Lindvall, ed. (Berlin: Springer-Verlag), pp. 63-74.

Widner, H. (1995). Transplantation of neuronal and nonneuronal cells into the brain. In *Immune reactions in the nervous system*, N. J. Rothwell, ed. (Oxford: Bios. Sci. Publ.), pp. 189-228.

Widner, H., and Brundin, P. (1988). Immunological aspects of grafting in the mammalian central nervous system. A review and speculative synthesis. *Brain Res. Rev* 13, 287-324.

Widner, H., and Brundin, P. (1993). Sequential intracerebral transplantation of allogeneic and syngeneic fetal dopamine-rich neuronal tissue in adult rats: Will the first graft be rejected? *Cell Trans.* 2, 307-317.

Widner, H., Brundin, P., Björklund, A., and Möller, E. (1989). Survival and immunogenicity of dissociated allogeneic fetal neural dopamine-rich grafts when implanted into the brains of adult mice. *Exp. Brain Res.* 76, 187-197.

Widner, H., Möller, G., and Johansson, B. B. (1988). Immune response in deep cervical lymph nodes and spleen in the mouse after antigen deposition in different intracerebral sites. *Scand. J. Immunol.* 28, 563-571.

Widner, H., Tetrad, J., Rehnström, S., Snow, B., Brundin, P., Gustavii, B., Bjorklund, A., Lindvall, O., and Langston, J. W. (1992). Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med* 327, 1556-63.

Williams, L. R., Varon, S., Peterson, G. M., Wictorin, K., Fischer, W., Bjorklund, A., and Gage, F. H. (1986). Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc. Natl. Acad. Sci. USA* 83, 9231-9235.

Winn, S. R., Hammang, J. P., Emerich, D. F., Lee, A., Palmiter, R. D., and Baetge, E. E. (1994). Polymer-encapsulated cells genetically modified to secrete human nerve growth factor promote the survival of axotomized septal cholinergic neurons. *Proc. Natl. Acad. Sci., USA* 91, 2324-2328.

Wolf, D., Richter-Landsberg, C., Short, M. P., Cepko, C., and Breakefield, X. O. (1988). Retrovirus-mediated gene transfer of beta-nerve growth factor into mouse pituitary line AtT-20. *Mol. Biol. Med.* 5, 43-59.

Wolff, J. A., Fisher, L. J., Xu, L., Jinnah, H. A., Langlais, P. J., Iuvone, P. M., O'Malley, K. L., Rosenberg, M. B., Shimohama, S., Friedmann, T., and Gage, F. H. (1989). Grafting of fibroblasts genetically modified to produce l-dopa in a rat model of Parkinson's disease. *Proc. Natl. Acad. Sci., USA* 86, 9011-9014.

Wood, M. J. A., Sloan, D. J., Dallman, M. J., and Charlton, H. M. (1992). A monoclonal antibody to the interleukin-2 receptor enhances the survival of neuronal allografts: a time-course study. *Neuroscience* 42, 409-418.

Wood, M. J. A., Sloan, D. J., Wood, K. J., and Charlton, H. M. (1996). Indefinite survival of neural xenografts induced with anti-CD4 monoclonal antibodies. *Neuroscience* 70, 775-789.

Wszolek, Z. K., Pfeiffer, B., Fulgham, J. R., Parisi, J. E., Thompson, B. M., Uitti, R. J., Calne, D. B., and Pfeiffer, R. F. (1995). Western Nebraska family (family D) with autosomal dominant parkinsonism. *Neurology* 45, 502-505.

Wuerthele, S. M., Freed, W. J., Olson, L., Morihisa, J., Spoor, L., Wyatt, R. J., and Hoffer, B. J. (1981). Effect of dopamine agonists and antagonists on the electrical activity of substantia nigra neurons transplanted into the lateral ventricle of the rat. *Exp. Brain Res.* 44, 1-10.

Yahr, M. D. (1977). Long-term levodopa in Parkinson's disease. *Lancet* 1, 706-707.

Yan, Q., Elliott, J., and Snider, W. D. (1992). Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy induced cell death. *Nature* 360, 753-755.

Young, A. B., Greenamyre, J. T., Hollingsworth, Z., Albin, R., D'Amato, C., Shoulson, I., and Penney, J. B. (1988). NMDA receptor losses in putamen from patients with Huntington's disease. *Science* 241, 981-983.

Yurek, D. M., Lu, W., Hipkens, S., and Wiegand, S. J. (1996). BDNF enhances the functional reinnervation of the striatum by grafted fetal dopamine neurons. *Exp. Neurol.* 137, 105-118.

Zetterström, T., Brundin, P., Gage, F. H., Sharp, T., Isacson, O., Dunnett, S. B., Ungerstedt, U., and Björklund, A. (1986). In vivo measurement of spontaneous release and metabolism of dopamine from intrastriatal nigral grafts using intracerebral dialysis. *Brain Res.* 362, 344-349.

Zhou, H., and Lund, R. D. (1993). Effects of the age of donor or host tissue on astrocyte migration from intracerebral xenografts of corpus callosum. *Exp. Neurol.* 122, 155-164.